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THE INFLUENCE OF SEX HORMONES ON THE TRANSAMINASES OF THE ACCESSORY SEX ORGANS OF THE MALE RAT*

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THE existence of multiple transaminating activities in the ventral prostate of the rat was recently demonstrated in this laboratory (Awapara and Seale, 1952). The role which the transamination reaction plays in intermediary metabolism of amino acids is not known precisely, but no doubt, it must be an important one. In addition to active transaminases, the ventral prostate possesses an unusually high concentration of free amino acids, a property not shared by the dorsal lobe. Some experiments on whole prostate carried out in this laboratory indicated that the concentration of free amino acids decreases considerably after castration and prostatic atrophy (Marvin and Awapara, 1949). Normal levels of free amino acids could be restored in the castrated animal by administering testosterone propionate.

The origin and fate of free amino acids in the ventral prostate are not known. Oxidative deamination of alanine and glutamic acid has been found to proceed at negligible rates whereas transamination was found to be very active (Barron and Huggins, 1946). It seems possible that most amino acids could transaminate to form glutamic acid and possibly glutamine. On the basis of this assumption the present study was initiated to appraise the influence of testosterone, estradiol and castration upon the transaminating activities of the prostate and seminal vesicle. The results re-

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ported here, indicate that sex hormones influence some activities but not others. Moreover, the change in enzymatic activity seems to be related to losses in protein, or some cellular component and not to a specific effect of the hormones investigated.

METHODS AND RESULTS

Free amino acids of the ventral and dorsal prostate were measured by pooling the organs from 12 animals. The preparation of protein-free filtrates and the microbiological assay of amino acids was carried out according to the methods described by Schurr and his associates (1950).

Only aspartic acid and alanine transaminases were measured because the quantity of tissue available was limited. The amount of glutamic acid formed from aspartic acid and alamine in the presence of α-ketoglutaric acid and tissue homogenate was measured by paper chromatography. The quantities of tissue, substrate and conditions used were identical to those previously reported (Awapara and Seale, 1952).

Concentration of Free Amino Acids in the Prostate

Extracts from ventral and dorsal prostates were prepared and the free amino acid composition of the extracts determined by two-dimensional paper chromatography. The ventral lobe was found to possess nearly all known amino acids and some other amino compounds (aminoethylphosphoric ester, taurine, glutathione, glutamine). The dorsal lobe was found to resemble other organs in its free amino acid distribution pattern. For quantitative assay of free amino acids, the organs from twelve animals were pooled in order to obtain a sufficient amount of material. The results recorded in table 1 confirm the chromatographic data. On the basis of amino nitrogen, the ventral prostate contains about three times as much free amino acids as the dorsal lobe.

Influence of Testosterone, Estradiol and Castration on Transaminases of the Prostate and Seminal Vesicle

Five groups of Wistar rats, ranging in weight from 280 to 300 grams, were used. Of necessity, the prostates of animals that have been castrated were pooled to obtain sufficient material. The prostates from three or four animals were pooled and six such pools used for the present work. In all other groups, a minimum of six animals were used and whenever the weight of the prostate was too small, the prostate from two animals of the same group were pooled. In all cases, six animals or six pools were used for each group. Castration was followed by a ten day period to allow prostatic atrophy. A second group of castrated animals received testosterone propionate in doses of 0.5 mg. per day for each animal for ten days, beginning the day following the operation. Three groups of whole animals were treated for ten days with (a) 0.25 ml of sesame oil, (b) 0.5 mg testosterone propionate per day for each animal and (c) 7.7 micrograms of α-estradiol per day for each animal. At the end of the tenth day the animals were fasted for 18 hours and sacrificed. The ventral and dorsal prostate, and the seminal vesicles were removed, weighed (the seminal vesicles were weighed, free of their contents) and transaminases determined in duplicate aliquots of tissue homogenates. The results reported in table 2, clearly point to significant changes in the transaminating activity of the accesory sex organs under different experimental conditions. The changes are more conspicuous in the ventral prostate than in the dorsal lobe or seminal vesicle.

DISCUSSION

The transaminating activity of the accessory sex organs follows a similar pattern of response to testosterone as other enzyme systems. Succinic dehydrogenase and phosphatases have been shown to decrease greatly in the prostate and seminal vesicle within eight days after castration (Davis, Meyer and McShan, 1949). Similarly, aspartic and alanine transaminases decrease considerably within ten days after castration in the ventral prostate and seminal vesicle but not in the dorsal prostate. In effect, there seems to be an increase in the aspartic transaminase of the dorsal lobe after castration. The dorsal lobe seems to be totally different from the anterior lobe not only in response to castration but in its amino acid composition as demonstrated in table 1. Differences in response of the dorsal and ventral prostate to injected testosterone have been reported by Steadman and Krichesky (1945) in terms of organ weight. These facts are in agreement with the present results.

TABLE 1. FREE AMINO ACID CONTENT OF PROSTATE
(In micrograms per gram of tissue)

Amino Acid	Ventral Lobe	Dorsal Lobe	Amino Acid	Ventral Lobe	Dorsal Lobe
Leucine	88	15	Glycine	21	20
Lysine	84	17	Glutamic Acid*	80	79
Valine	41	10	Alanine	14	11
Phenylalanine	47	8	Aspartic Acid*	20	13
Serine	58	18	Tryptophane	13	1
Histidine	37	2	Arginine	44	12
Proline	49	3	Cystine	16	10
Isoleucine	28	—	Tyrosine	36	15
Methionine	8	1	Threonine	66	—

* Determined by quantitative paper chromatography (Awapara, 1949).

Changes in enzymatic activity elicited by the action of testosterone, castration or estradiol would not necessarily entail a direct effect upon the enzyme. The changes in the alanine transaminase in the ventral prostate appear to be related to the losses of some cellular components. It is interesting to note that the alanine transaminase decreases in proportion to weight losses, brought on either by castration or estrogen treatment. On the other hand aspartic transaminase changes slightly and even increases when the losses in weight are relatively small. Evidently alanine transaminase is associated with a more labile type of protein than the aspartic transaminase. Since the values reported in table 2 are given per unit weight it is not surprising to find actually an increase in aspartic transaminase in the ventral prostate after estrogen treatment. If certain cellular components not associated with this enzyme are lost, a concentra-

tion of the enzyme would occur. Unpublished data from this laboratory supports the assumption that alanine and aspartic transaminases are associated with different types of protein. Homogenates of ventral prostate were centrifuged and the insoluble residue separated from the soluble protein. Nearly all the aspartic transaminase was associated with the soluble fraction and most of the alanine transaminase with the insoluble residue.

On the basis of the present investigation, it is impossible to postulate a mechanism for the hormonal control of amino acids level in the prostate. If transaminases control the free amino acids level in the prostate, then after castration an increase rather than a decrease in free amino acids should

TABLE 2. INFLUENCE OF SEX HORMONES ON THE TRANSAMINASES OF ACCESSORY SEX ORGANS OF THE RAT

Treatment	Ventral prostate			Dorsal prostate			Seminal vesicle		
	Weight mg.	Aspartic Transam.	Alanine Transam.	Weight mg.	Aspartic Transam.	Alanine Transam.	Weight mg.	Aspartic Transam.	Alanine Transam.
Normal Control	283 ± 42.0	158 ± 5.8	119 ± 5.9	170 ± 18.5	128 ± 4.1	14 ± 3.6	208 ± 18.3	162 ± 3.0	31 ± 4.1
Testosterone	428 ± 22.7	145 ± 2.7	105 ± 10.0	266 ± 16.0	131 ± 3.7	12 ± 2.4	324 ± 16.0	201 ± 11.6	27 ± 2.0
Estradiol	147 ± 14.0	180 ± 4.5	73 ± 16.7	144 ± 14.7	148 ± 8.0	10 ± 3.0	151 ± 11.7	164 ± 12.7	23 ± 7.8
Castration	59 ± 2.1	117 ± 1.4	19 ± 1.2	87 ± 3.3	138 ± 2.6	8 ± 2.3	140 ± 7.2	133 ± 0.7	10 ± 2.0
Castration and Testosterone	335 ± 18.7	147 ± 7.0	105 ± 6.9	204 ± 11.3	122 ± 9.7	20 ± 5.0	302 ± 14.6	180 ± 7.4	40 ± 4.2

Arithmetic mean of values from six animals plus or minus the standard error.

result, since the transfer of amino groups to α-ketoglutarate has been slowed considerably. This is not the case.

One likely explanation for the high concentration of free amino acids in the normal ventral prostate may be found in the high rate of citric acid synthesis in that organ. It is well known that the ventral prostate of the rat possesses one of the highest concentrations of citrate (100–120 mg /100). On the other hand, citric acid is not utilized in the prostate (Huggins and Barron, 1946). In the absence of citrate oxidation, α-ketoglutarate cannot be formed, thus creating a deficiency. Inasmuch as α-ketoglutaric acid is one of the most active amino group acceptors, it seems plausible that in its absence little deamination can take place. Recent experiments (Awapara, in press) have demonstrated that upon incubation of ventral prostate with α-ketoglutaric acid a rapid decrease in the concentration of most amino acids occur.

The only possible pathway for the deamination of amino acids in the prostate seems to be by transamination. Actually little α-ketoglutaric acid

is needed to deaminate large concentrations of amino acids; the glutamic acid formed can be deaminated readily by glutamic dehydrogenase which is present in most tissues and the resulting α-ketoglutaric acid could be recycled. A scheme like this was proposed and supported by some experimental data by Braunstein (1947).

In conclusion, it must be stated that the role of transaminases in the accessory sex organs must be an important one. They act, in all probability, in the deamination of amino acids. Changes in transaminase activity in the accessory genitalia after treatment with sex hormones or following castration seem to be secondary effects resulting from the loss of certain cellular components.

SUMMARY

1. The free amino acid composition of both ventral and dorsal prostate of the rat was determined by chromatographic and microbiological methods. The ventral prostate was found to possess unusually high amounts of free amino acids, a property not shared by the dorsal lobe.

2. The effect of castration, and treatment with testosterone and estradiol on the activities of aspartic and alanine transaminases of the prostate and seminal vesicle was investigated. Significant changes were observed in the transaminating activities of the ventral prostate. These changes seem to be correlated to the loss of some cellular components.

The author wishes to acknowledge the valuable assistance of Mrs. Leota Skirvin.

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THE URINARY PHOSPHATE EXCRETION FACTOR OF PARATHYROID GLAND EXTRACTS: A HORMONE OR AN ARTEFACT?[1]

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TWO hypotheses have been advanced in explanation of the parathyroid hormone control over the level of ionized calcium in the tissue fluids. One is that the hormone induces a release of calcium by a direct stimulating action on the bones (Thomson and Collip, 1932); chemical changes, as reflected by the varying concentrations of calcium and of phosphorus in the serum and in the urine, are considered secondary manifestations. According to the other hypothesis, the hormone acts primarily on the electrolyte equilibria of the body fluids in such a manner as to increase their solvent power for bone salts; bone dissolution follows as a secondary phenomenon (Albright *et al.*, 1929; Albright and Reifenstein, 1948). In brief, the sequence of events is supposedly: (1) the hormone acts on tissue fluid phosphate rendering it more readily excretable by the kidney; (2) serum phosphate is thereby decreased resulting in tissue fluid undersaturation with respect to some equilibrium constant regulating serum calcium and phosphate concentrations; (3) solution of bone salts occurs leading to an accumulation of calcium in the serum.

The present findings offer negative support only to the bone cell hypothesis of Collip and his associates in that they are in no way irreconcilable with it; however, they do appear irreconcilable with the chemical solution hypothesis of Albright and co-workers, especially with regard to the primary importance of kidney function and tissue fluid phosphate.

The chemical solution concept implicitly assumes: that the parathyroid hormone would be relatively inactive (a) in the absence of the kidneys, or (b) under any (other) circumstances leading to an accumulation of phosphate in the tissue fluids; (c) that a continuously inverse correlation obtains between the calcium and the phosphorus of serum. Any evidence that would invalidate the first assumption would do likewise to the second; the third assumption is integral to the entire mechanism and its invalidation would render the hypothesis untenable.

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[1] Supported by the Otho S. A. Sprague Memorial Institute.

These assumptions are decidedly in question. A qualitative action of parathyroid hormone in the nephrectomized animal has been frequently reported (see review by Greep, 1948); in confirmation, we have shown that a quantitative response can be readily elicited in the bilaterally nephrectomized dog (Stewart and Bowen, 1951). That the hormone can exert its action in the presence of a rapidly mounting serum phosphate must therefore be accepted also. With regard to the third assumption, there appears to be no direct experimental evidence to show the existence of a continuous inverse relationship between the calcium and the phosphorus of serum; to the contrary, there is much evidence to indicate that these two constituents of serum can vary independently of each other over wide ranges of concentration and in directions not always predictable (Collip, 1926; Thomson and Collip, 1932; Thomson and Pugsley, 1932; Logan, 1938).

There is one bit of evidence considered by some to be supportive of the chemical solution hypothesis that cannot be lightly dismissed. This is the rather consistent increase in urinary phosphate excretion that immediately follows the injection of parathyroid gland extracts (Albright *et al.*, 1929). We have sought an alternate explanation for this phenomenon.

We have achieved a biological separation of the calcium mobilizing and the phosphate excretion activities of parathyroid gland extracts; the latter is also present in similarly prepared extracts of spleen and of thymus. Neither of these two activities is dependent on the diuresis reportedly attending parathyroid extract administration. In addition, we have confirmed our previous finding that the calcium mobilizing action of parathyroid hormone is not primarily contingent on kidney function.

METHODS

The chemical methods and procedures involving the use of animals were, when applicable, essentially those previously described (Stewart and Bowen, 1951). For the phosphate excretion studies the animals were kept under sodium pentobarbital anesthesia to prevent voluntary excretion of urine and to maintain basal conditions. Urine was collected hourly by means of an indwelling catheter and the bladder was rinsed each time with saline to ensure complete collections. The samples were acidified with 1 ml. conc. HCl per 100 ml. to solubilize the inorganic salts and then filtered. Serum and urinary inorganic phosphate were determined by the method of Kuttner and Lichtenstein (1930) on the 5 per cent trichloroacetic acid filtrates. Sodium citrate was injected intravenously as an 0.075 M solution at a dose of 5 ml. per kg. of body weight and at a rate of 5 ml. per minute. Owing to the low tolerance of the parathyroidectomized animals these received 3 ml. per kg. of body weight.

The parathyroid extract (Lilly)[2] contained 100 U.S.P. Parathyroid Units per ml. and was injected intravenously. Inactivation of the extract was secured by the addition

[2] Our thanks are due the Lilly Research Laboratories for their generous supply of extract and for the instructions concerning the method of inactivation employed.

of one part formaldehyde to one hundred parts of extract followed by incubation for four days at room temperature. Alcohol:ether (3:1) were then added, the proteins centrifuged down and redissolved in distilled water for injection. This procedure results in practically complete inactivation of the calcium mobilizing principle of the extract. The extracts of spleen and of thymus (beef)[3] were prepared by the method of L'Heureux, Tepperman and Wilhelmi (1947) since this was reported to yield a material with high nitrogen potency as measured by the decrease in serum inorganic phosphate in the rat. For purposes of injection it was necessary to suspend the desiccated material in distilled water with the addition of dilute HCl to approximately pH 3.5 to bring about solution. This procedure was required for the solution of some of the preparations used by L'Heureux *et al.* (personal communication). The material was injected intravenously at a dose of 100 mg. in a final volume of 15 ml., although doses one-fifth this size exhibited phosphate excretion activity in preliminary studies.

A total of 12 dogs was used in the 35 trials of the phosphate excretion studies. This limitation in the number of animals, necessitated by the problem of supply, might have resulted in a decrease in the variability of response; however, it would be disadvantageous in the event that multiple injections should have resulted in the building up of a tolerance for the extracts since there would tend to be a reduction in the differences between the experimental means. Thyroparathyroidectomized dogs were employed for one trial in treatment H and for four trials in treatment I (Figure 2).

RESULTS AND DISCUSSION

(1) *The response of bilaterally nephrectomized dogs to sodium citrate*

The stimulus for parathyroid hormone production in the intact animal appears to be an abnormally low level of ionized calcium in the plasma (Patt and Luckhardt, 1942). Whereas in our previous study sodium oxalate was employed to reduce this fraction of blood calcium, the relatively innocuous sodium citrate was used in the present one. The results are depicted graphically in Figure 1.

Curve A shows the response of a dog injected with sodium citrate at 0, 1, 2, 4, 6, 8 and 10 hours. The increase of 14.1 mg. per cent in serum calcium within 10 hours reflects a high degree of parathyroid hormone activity. In an experiment in which two dogs received citrate every 2 hours for 26 hours (Curve B) a mean increase of 6.9 mg. per cent was obtained. The difference in origin and shape of the two curves suggests the existence of a state of hyperparathyroidism in the first case. Of interest is the average 84 per cent increase in serum phosphate observed in these three animals within 10 hours. The graded nature of the response is indicated by the reaction of the two animals that received a single injection of citrate at zero time (Curve C); a mean increase in serum calcium of 2.6 mg. per cent was noted. The quantitation of these responses in terms of parathy-

[3] We wish to thank the Armour Laboratories for supplying the frozen bovine spleen and thymus.

roid gland extract is not possible since the normal animal rarely exhibits any change in serum calcium levels following citrate administration; the response is mirrored solely by an increased urinary excretion of calcium (unpublished data).

The elimination of the response by parathyroidectomy is shown for five dogs (Curve D). Only one of these tolerated more than a single injection of citrate; those that survived longer than 5 hours showed no change in serum calcium levels. Several other animals died immediately following the

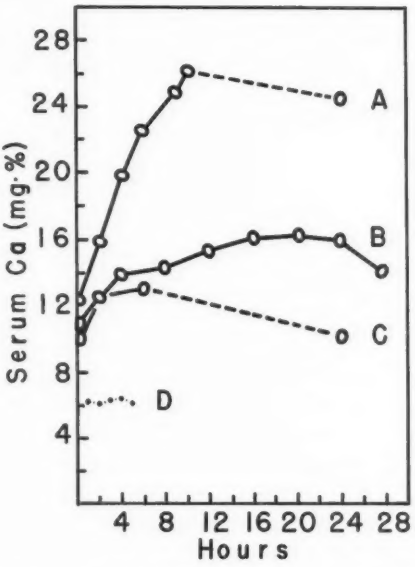

FIG. 1. Serum calcium curves for nephrectomized dogs receiving sodium citrate intravenously. Animals in Curve D were also thyroparathyroidectomized.

administration of the citrate. On the basis of our oxalate tolerance studies the reversibility, by the use of parathyroid gland extracts, of the elimination of the response in parathyroidectomized animals is assumed since reversibility is possible in the animal with kidneys intact (unpublished data).

The extent of the decline in tissue fluid ionized calcium following citrate treatment is evident from the increased neuromuscular excitability manifested in each animal throughout the entire experimental period. The consequent increase in parathyroid hormone production and activity is reflected in the serum calcium response curves. The results are perfectly definite, especially in relation to their bearing on the chemical solution hy-

pothesis: endogenous parathyroid hormone mobilizes calcium in the absence of the kidneys; furthermore, this mobilization occurs in the presence of a rapidly increasing serum inorganic phosphate. Since it is a generally accepted fact that the hormone can mobilize calcium in the presence of a high serum calcium (as in hyperparathyroidism) even with an accompanying high serum phosphate (as in parathyroid hormone overdosage), the difficulty of reconciling the hypothesis with the data is apparent.

(2) *The phosphate excretion factor*

The factor under consideration is that which rather consistently brings about a marked increase in the urinary excretion of inorganic phosphate within the first hour following the administration of parathyroid gland extracts.

Urine samples for phosphate analysis were collected hourly for a three hour control period and for the post-injection period. Since the urinary phosphate of the second group (treatment I) returned to control levels the third hour after injection, the data for all groups include only a three hour post-injection period.[4] These data are shown in reduced form in Figure 2. Parathyroid gland extract (treatment H), in doses ranging from 1 to 10 ml., produced the greatest increase in phosphate excretion as would be anticipated were phosphate mobilized along with calcium as a result of hormone-stimulated bone dissolution. In similar doses, the inactivated extract (treatment I) also induced urinary phosphate excretion. The effect on serum phosphate was variable and not statistically significant. The absence of any change in serum calcium demonstrates the completeness of the inactivation and strongly argues the separate entity of these two factors. The latter receives substantiation by the fact that both spleen extract (treatment S) and thymus extract (treatment T) also produced a significant increase in the excretion of urinary phosphate. Controls showed no change (treatment C).

The biological separation of the calcium mobilizing and the phosphate excretion activities present in parathyroid gland extracts, with the demonstration of the latter activity in similarly prepared extracts of nonparathyroid tissues, categorizes the phosphate excretion factor as a pharmacologically active tissue degradation product. The sudden out-

[4] This return of the urinary phosphate excretion to normal within three hours is similar to that reported by Albright and his associates (1942). Their patient with idiopathic hypoparathyroidism responded to an intravenous injection of 200 units of parathyroid gland extract with a urinary phosphate excretion of 313 and 120 mg. of phosphorus on the first and second hours respectively after the injection; by the third hour the phosphate excretion had returned to the pre-injection level of around 20 mg. per hour.

pouring of phosphate immediately subsequent to parathyroid extract administration is therefore to be considered, not a phenomenon intimately associated with hormonal activity, but merely a response to an artefact.

Incidental to the present study was the fact that in no instance did we observe any increase in the volume of urine due, perhaps, to the antidiuretic nature of the anesthetic, although some of the post-injection periods extended to 7 hours. The reported diuretic action of parathyroid gland extracts (Shelling *et al.*, 1938) is therefore not fundamental to either

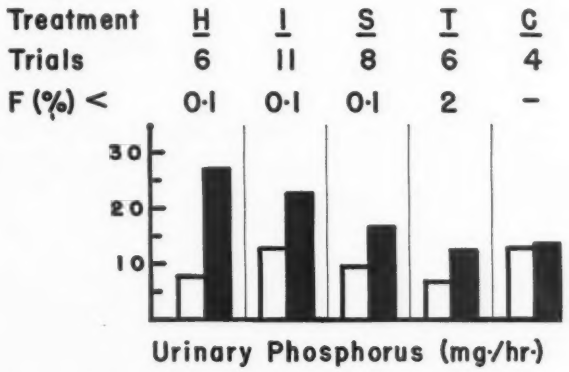

FIG. 2. Hourly excretion of urinary phosphorus in dogs for a three hour control period (plain bar) and a three hour post-injection period (solid bar). Treatments were: H—hormone; I—inactivated hormone; S—spleen extract; T—thymus extract; C—controls with no injection. Trials indicate number of experiments. F(%) denotes the "per cent point" of the variance ratio in an analysis of variance to determine the significance of difference between the subsample means within each treatment only.

the calcium mobilizing or the phosphate excretion activity of the extracts studied.

SUMMARY

Multiple and single intravenous injections of sodium citrate into bilaterally nephrectomized dogs caused parathyroid gland stimulation with a resultant rapid and marked hypercalcemia. Thyroparathyroidectomized animals could tolerate only small amounts of citrate and failed to manifest any change in serum calcium. These observations confirm our previous findings that the calcium mobilizing activity of endogenously produced parathyroid hormone is not contingent on kidney function.

That the intravenous injection of parathyroid gland extract causes an immediate increase in the urinary excretion of inorganic phosphate has

been confirmed in the dog. The artefactitious nature of the factor producing this phenomenon is established by the demonstration of its presence (1) in formaldehyde inactivated parathyroid gland extracts devoid of calcium mobilizing activity, and (2) in extracts of spleen and of thymus (beef) prepared by a process similar to that for the preparation of active parathyroid gland extracts.

In no instance was a diuresis noted; the reported diuretic activity of parathyroid gland extracts is therefore of no fundamental importance to either the calcium mobilizing or the phosphate excretion activity of the extracts studied.

The chemical solution hypothesis of parathyroid hormone action is discussed from the standpoint of its irreconcilability with existent data.

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THE EFFECTS OF VITAMIN A AND REICHSTEIN'S COMPOUND L ON THE THYROID, ADRENAL AND LYMPHOID SYSTEMS OF THE RAT*

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WE HAVE previously reported (1950–1951) that various steroids influence thyroid function in the rat as measured by radioactive iodine collection. Moreover, some of these compounds have marked effects on the adrenal glands and lymphoid tissue (Money, Fager and Rawson, 1952). One of the steroids we investigated was Reichstein's Compound L (3β-acetoxy-17-hydroxyallo-pregnan-20-one). Under the experimental conditions used in the above studies, this compound decreased the I^{131} collected by the thyroid, produced some hypertrophy of the adrenals, decreased thymus weight, and enlarged mesenteric lymph nodes. Biochemical studies done on these animals indicated that a relationship might exist between this steroid and the circulating level of vitamin A (Bodansky and Markardt, 1951).

Since Compound L appeared to influence vitamin A concentration in various tissues, it was decided to investigate the relationship of known vitamin A intake on the previously observed effects of Compound L. This report is the result of these studies.

MATERIALS AND METHODS

One hundred and thirty-seven immature male rats of the Sprague-Dawley strain were used in this study. The animals (weight range 32–53 grams) were divided into twelve approximately equal groups so that the average body weight was 41–45 grams. They were placed on a U.S.P. XIII vitamin A free diet (obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio). Six groups of rats received either 0, 2, 10, 25, 50, or 100 i.u. of vitamin A daily (by oral administration) and a subcutaneous injection of Com-

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pound L.* The dosage of Compound L was graduated in accordance with the average gain in body weight. For the first seven days this dose was 5 mg/day, for the next 12 days 7 mg, and 10 mg/day for the rest of the study.

U.S.P. vitamin A reference standard containing 10,000 i.u. of vitamin A as crystalline vitamin A acetate per gram of cottonseed oil was used as a supplement. It was diluted with cottonseed oil so that the daily dose was 0.1 cc and fed to each animal through a syringe. The control animals received only vitamin A and 0.1 cc subcutaneously of a solution containing 1 per cent acasia and 0.01 per cent sodium ethyl mercurithiosalicylate.

Throughout the remainder of this paper the groups of animals will be referred to as group A0, A2, A10, A25, A50, A100, or A0L, A2L, A10L, etc. The number indicates the daily dose of vitamin A. The presence of "L" in the code indicates that the group received Compound L.

Groups A0, A0L, A2, A2L, A10, and A10L received their last treatment on the morning of the thirty-ninth day. They were fasted from 4 P.M. of that day to the time of autopsy on day 40. The remaining groups received their last treatment on the fortieth day, were starved overnight, and killed on the forty-first day.

Twenty-four hours before autopsy all animals were given 1 μc of carrier-free I^{131} intraperitoneally. At the time of autopsy the rats were given a small dose of sodium pentobarbital and exsanguinated from the dorsal aorta.†

Organs were removed, dissected free of fat and connective tissue and weighed on a Roller-Smith balance. They were then placed in 10 cc vials containing 5 cc of Bouin's Solution. The counting of thyroidal I^{131} was done in a Texas Company gamma-ray Geiger-Müller tube. The I^{131} in the thyroid tissue has been expressed as a percentage of the injected solution, an aliquot of which was measured at the same time and under the same conditions as the glands.

The information we obtained is presented in Table 1.

EXPERIMENTAL RESULTS

I. *Changes in Body Weight.* Table 1 indicates that there is a rough correlation between the growth of the animals and the intake of vitamin A. With low levels of this vitamin, animals failed to gain as much weight as those receiving the higher doses. The groups which received 10 units or more of vitamin A daily had average body weights which were essentially the same.

The addition of Compound L to animals receiving various amounts of vitamin A produced a decrease in average body weight in all groups given 10 units or more of the vitamin daily. With the lower doses of vitamin A (groups A0L and A2L) there was no loss in body weight produced by the Compound L injections. Moreover, these Compound L treated animals looked in better condition than their controls (groups A0 and A2) and

* Compound L was kindly furnished by Dr. Irving Sollins of the Syntex Company.

† Studies done on the plasma vitamin A as well as levels in the kidney and liver are reported in a separate publication by Bodansky and Markardt (J. Biol. Chem. **190**: 83, 1951).

indications of vitamin A deficiency were less marked (Bodansky and Markardt, 1951).

II. *Changes in thyroid weight.* No consistent pattern appeared in the absolute weight of the thyroid glands (see Table 1). Neither the differ-

TABLE 1

Group	Body wt. (Gm.)	Thyroid wt. (Mg.)	%I^{131}/Thyroid	Adrenals (Mg.)	Thymus (Mg.)	Lymph nodes (Mg.)
A0	128	13.5	56.53	28.2	317.4	12.76
A0L	129	11.9	45.10	23.0	310.4	27.13
A2	132	11.3	55.73	28.7	350.7	16.86
A2L	140	12.9	45.47	23.5	337.4	33.71
A10	156	13.7	57.77	23.4	385.8	19.34
A10L	135	13.4	48.75	22.8	252.9	32.07
A25	159	11.9	36.05	32.1	402.9	22.00
A25L	141	11.8	30.50	26.6	295.7	31.05
A50	156	13.7	48.43	29.9	436.7	25.58
A50L	144	12.9	32.14	28.6	291.7	33.56
A100	152	16.3	50.46	29.2	368.2	30.87
A100L	134	10.7	28.80	26.7	288.1	31.42

* "P" values between various groups

A0-A25 = <0.05 A0-A10 = <0.01	A100-A100L = <0.01	A0L-A25L A0L-A50L A0L-A100L A50-A50L A100-A100L = <0.01	A0L-A100L A0-A10 = <0.02 A0-A0L A0L-A50L A2-A2L A25-A25L = <0.01	A100-A00L = <0.05 A25-A25L = <0.02 A10-A10L A50-A50L = <0.01	A0-A25 A25-A25L = <0.05 A0-A50 A0-A100 A0-A0L A2-A2L A10-A10L = <0.01

* P values calculated according to method of Fisher, 1941.

ences nor the direction of change was generally marked among the various groups. The only marked significant difference found was between groups A100 (thyroids, 16.2 mg.) and A100L (thyroids, 10.7 mg.). The p value between these groups was <0.01. Whether this was fortuitous, or an indication that Compound L treatment is more effective with high intakes of vitamin A is unknown.

III. *Changes in the collection of I^{131}.* The absolute amount of I^{131} present in the thyroids of these animals is shown in Table 1. It appears that vitamin A intakes of 0, 2, or 10 units daily did not change the I^{131} uptake. Doses greater than this depressed, to various degrees, the amount of

radioactivity found in the gland. The greatest decrease in I^{131} pickup was found in the group which received 25 units of vitamin A daily ($p = <0.01$).

Compound L administration decreased in all groups the thyroid I^{131} collection below the percentage found with vitamin A alone. The greatest decrease was found in animals which received 50 or 100 units of vitamin A and Compound L.

IV. *The changes in adrenal weight.* No consistent trend was found in the changes in adrenal weight in animals receiving only vitamin A. The smallest adrenals were in animals receiving 10 units of vitamin A daily (p between A0 and A10 <0.02), while the largest adrenals were found in Group A25 which received 25 units daily.

Compound L decreased the weight of the adrenal glands in all groups of animals below that found with the vitamin A treatment alone. Groups A10 and A10L had adrenals which were, however, essentially of the same weight. It is interesting that the adrenals of animals receiving the lower doses of vitamin A and Compound L (Groups A0L, A2L, and A10L) were smaller than the adrenals of groups A25L, A50L, and A100L which received higher doses of the vitamin. Although statistically most of these differences are not significant, the results suggest that high intake of vitamin A alone or with Compound L produces hypertrophy of the adrenals.

V. *Changes in thymus weight.* Table 1 suggests that there is a correlation between vitamin A intake and the thymus weight. This appears to be true for every group except A100. This might indicate that this high dose of the vitamin had a toxic effect on the thymic tissue. When one considers the thymus-body weight ratio, however, it is apparent that no direct correlation exists between vitamin A intake and thymus weight.

Treatment of animals with Compound L produced a depression of the thymus weight. This decrease is less marked in the rats which received 0 or 2 units of vitamin A daily. The thymus-body weight ratio of Compound L treated animals is lower than controls (vitamin A alone) in all groups. Groups receiving 10, 25, and 50 units of A are significantly lower ($p = <0.05$) than the corresponding animals treated only with vitamin A. Moreover, the thymus-body weight ratio is essentially the same in all groups receiving 10 or more units of vitamin A and Compound L.

VI. *Changes in lymph nodes.* Table 1 indicates that there is a definite relationship between lymph node weight and vitamin A intake. Moreover, when the lymph node-body weight ratio is calculated, this relationship still holds.

The administration of Compound L to animals receiving various amounts of vitamin A produced essentially the same lymph node weight regardless of vitamin intake. The most striking fact was that the marked

differences in lymph node weight seen with lower doses of vitamin A disappeared when the doses were increased.

DISCUSSION

Various studies have indicated a relationship between the function of the endocrine system and vitamins. For example, folic acid appears to be essential for certain actions of estrogens (Hertz and Tullner, 1949). Experimentally, pantothenic acid and riboflavin have been shown to play a role in maintaining normal function of the adrenal cortex (Deane and McKibbin, 1946; Gaunt, Liling, and Mushett, 1946; Dumm, Ovando, Roth and Ralli, 1949). Vitamin C has been reported to be involved in the action of the thyroid stimulating hormone on the thyroid as well as playing a role in adrenal physiology (Marine, Baumann and Rosen, 1934).

There is a general lack of agreement on the role of vitamin A in thyroid function. Many reports have indicated that an antagonism exists between the thyroid hormone and this vitamin. Recent reports conclude that vitamin A plays a definite role in thyroid physiology and is a promising agent in the treatment of hyperthyroidism (Simkins, 1947). Lipsett and Winzler (1947) have shown that vitamin A deficiency interferes with the formation of thyroxine and that iodine metabolism is abnormal in vitamin A deficient animals. With our methods we have not confirmed the many reports that high doses of vitamin A inhibit the activity of the thyroid. The greatest difference we have observed in depression of I^{131} collection occurred in animals receiving 25 units of vitamin A daily. This dosage is not far from that reported as the maintenance dose of vitamin A for the rat (Lewis, Bodansky, Falk and McGuire, 1942).

We have previously reported that Compound L decreases the percentage of I^{131} collected by the thyroid gland (1950–1951). The mechanism of this depression is still unknown. We have, however, unpublished data which indicates that Compound L does not change the percentage of the total butanol soluble I^{131} found in the thyroid. Future studies may determine which thyroid iodine fraction is decreased.

Adrenal size was not significantly changed with different intakes of vitamin A. Compound L produced slight adrenal weight decrease in all groups. However, the adrenal atrophy induced by Compound L treatment was not striking in the above experiments. Our previous studies (1952) with this steroid have indicated that in adult animals its administration produced adrenal enlargement. The meaning of this is somewhat obscure, as we have unpublished data that Compound L, under certain conditions, may produce all the other observed responses without changing the adrenal weight.

Vitamin A appears to induce thymic hypertrophy with increase in dosage. However, when calculations are made for changes in body weight, the organ-body ratio is not markedly changed. We have shown earlier (1952) that Compound L produced a decrease in the thymus weight. Whether this is a direct effect, or mediated through the adrenals or by some other mechanism, is unknown.

Our observations suggest that a direct relationship exists between vitamin A intake and mesenteric lymph node weight. That Compound L administration produces hypertrophy of lymph nodes has been reported earlier (1952). From the observations above, however, it would suggest that this occurs only when animals are receiving smaller intakes of vitamin A than is usually found in laboratory food.

The mechanism of Compound L action in producing the above responses is unknown. One has, however, to consider several possibilities. First, it is possible that this steroid interferes with the regulation of vitamin A. This is suggested by the report of Bodansky and Markardt (1951), who found the plasma vitamin A level reduced in the animals used above. Secondly Compound L may be acting partly via the pituitary and adrenal glands in a manner still not understood. This might account for the adrenal hypertrophy and thymus atrophy we have observed. It fails, however, to account for lymph node enlargement. Thirdly the effect may be mediated by the thyroid. That the thyroid is related to lymphoid tissue has been frequently suggested. The work of Marder (1949) offers some support to this theory. He showed that the lymph nodes and thymus are increased in weight in adrenalectomized mice treated with thyroxine, whereas in intact animals treated with thyroxine the lymph nodes are increased and the thymus decreased. Finally the effects we have observed after administration of Compound L may be nonspecific in nature. All of these possibilities are being considered and further studies are under way to elucidate the mechanism of the action of Compound L.

SUMMARY

Immature male rats were maintained on a vitamin A deficient diet for about five and one half weeks. Half of the animals received either 0, 2, 10, 25, 50, or 100 i.u. of vitamin A daily and injections of Compound L. The other groups received only the vitamin A. At autopsy, weights of the thyroid, adrenal, thymus, and mesenteric lymph nodes, and thyroidal I^{131} collection were determined.

The results indicate that both compounds decrease the collection of I^{131} found in the thyroid. Compound L, however, produced a more marked depression than high doses of vitamin A. Low or high intakes of vitamin A slightly increased adrenal weight. Compound L was found to increase

adrenal weight with the higher levels of Vitamin A. Increased thymus weight was observed with increased intake of vitamin A. Compound L not only prevented this but decreased thymus size. There appears to be a direct correlation between vitamin A intake and lymph node size. Compound L produced marked hypertrophy of lymph nodes except with the higher levels of vitamin A.

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ACTION OF ASCORBIC ACID ON THE RESPONSE OF THE PITUITARY-ADRENAL AXIS IN THE GUINEA PIG

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PREVIOUS work indicated that ascorbic acid pretreatment to rats (Bacchus *et al.*, 1951; Bacchus, 1951), and to mice (Bacchus and Altszuler, 1951) prevented the eosinopenia characteristic of the alarm reaction. Moreover, it was demonstrated that the eosinophil response of the ascorbic acid-pretreated rat to epinephrin was similar to the response of the adrenalectomized rat to the same stressor. It was shown that the vitamin pretreatment did not block the eosinophil response to adrenocorticotrophin (Bacchus and Altszuler, 1951). Those data were interpreted as indicating that the vitamin acted by blocking the pituitary-adrenal axis at a pituitary or a pre-pituitary level, hence preventing the release of adrenocorticotrophin by epinephrin, and thereby blocking the increased release of corticosteroids from the adrenal cortex. It was recognized, however, that the blockage of response to epinephrin could be due to a specific ascorbic acid-epinephrin antagonism.

This paper reports data on experiments which were planned to determine whether the vitamin possesses some specific anti-epinephrin action, and whether the vitamin *per se* alters the hematologic relations.

MATERIALS AND METHODS

These experiments were conducted on adult male guinea pigs averaging 480 grams. All animals were kept in laboratory quarters of fairly constant temperature and humidity, and were fed with Purina Rabbit Chow, fresh greens, and tap water. The adrenalectomized animals used in experiment IV received 0.9% NaCl in place of tap water. The method used for determining adrenal cholesterol content was modified from Schoenheimer and Sperry (1934), the method for blood glucose from Folin (1928). Total and differential leukocyte counts were made by a modification of Randolph's (1944) method in which eosin was substituted for phloxine. This method was found to be superior to that used by Thorn (1950) for eosinophil counting, and in addition afforded the total and differential counts on the same preparation. Blood for the sugar determinations and for leukocyte counts was obtained from ether-anesthetized animals from the toe. The nail was cut off just distal to the root, and blood drawn from this wound. Adequate samples of blood were rapidly collected without the use of anti-coagulants.

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EXPERIMENT DESIGNS AND RESULTS

Experiment I: This experiment was designed to determine the effect of ascorbic acid injection on the blood leukocyte count, blood glucose concentration, and adrenal cholesterol content of intact guinea pigs. This experiment became necessary in view of the unpublished data of Therien (quoted by Dugal, 1951) that the vitamin induced signs of the alarm reaction in mice and guinea pigs. Observations were made on 11 guinea pigs which received single intraperitoneal injections of ascorbic acid (100 mg./100 g. body weight, of Vitamin C, Injectable, Sodium ascorbate, Roche). Eight control guinea pigs received single intraperitoneal injec-

TABLE 1. HEMATOLOGIC AND ADRENAL CHOLESTEROL RESPONSES TO ASCORBIC ACID AND TO SALINE IN INTACT GUINEA PIGS

Treatments	Ascorbic acid	Saline
No. of animals	11	8
Leukocytes*	+1,400 ±1,400	−700 ±1,000
Lymphocytes (Absolute)*	− 200 ±700	−400 ±500
Neutrophils (Absolute)*	+1,500 ±900	−350 ±700
Eosinophils*	+ 20 ±20	+ 4 ±23
Eosinophils % change	+ 13%	+ 1.5%
Blood Glucose (mg./100 cc.)		
M.D. S.E.†	+ 10.3 ±44.0	+8.0
% change	+ 3.9%	
Adrenal Cholesterol		
(mg./100 mg. adrenal)	1.302 ±0.167	1.300 ±0.181
(Mean ±S.E.)	(n=7)	(n=4)
mg./100 g. body wt.	0.210 ±0.042	0.228 ±0.061
(Mean ±S.E.)		

* Mean differences: number of cells/cu. mm. before, and 3½ hours after, injections. (±S.E.)

† Mean differences: mg. glucose/100 cc. blood before, and 3½ hours after, injections. (±S.E.)

tions of saline solution. Blood for leukocyte counts and glucose determinations was collected just prior to, and 3½ hours after, the injection. The adrenals of seven of the vitamin-treated, and four of the control, animals were immediately removed for cholesterol determinations.

Results: The data obtained are presented in Table 1. Ascorbic acid failed to alter the hematologic pattern and failed to depress markedly the adrenal cholesterol content. The results indicate that the vitamin, even in such massive doses, fails to elicit the alarm reaction in the guinea pig.

Experiment II: In this experiment the action of ascorbic acid treatment on the hematologic response to epinephrin was investigated. Nine guinea pigs were injected intraperitoneally with ascorbic acid (100 mg./100 g. body weight) and at the same time recieved single subcutaneous injections of 0.02 mg. epinephrin hydrochloride per 100 g. body weight. Eight control animals received saline instead of ascorbic acid. Blood samples were

taken just prior to, and 3½ hours after, the injection. The adrenal cholesterol content was not determined.

Results: The data are presented in Table 2. It is observed that epinephrin in the control animals induced a significant increase in the circulating numbers of leukocytes, and neutrophils, associated with significant reductions in the numbers of circulating lymphocytes, and eosinophils. The blood glucose levels of these animals was significantly increased 3½ hours after the injections. On the other hand, the animals which received ascorbic acid plus epinephrin failed to show the lymphopenia and eosinopenia observed in the control group of animals. It became evident that although

TABLE 2. HEMATOLOGIC RESPONSE TO EPINEPHRIN (0.02 MG./100 G. B.W.) IN ASCORBIC ACID-TREATED, AND SALINE-TREATED, GUINEA PIGS

Treatments	Ascorbic acid	Saline
No. of animals	9	8
Leukocytes (Total)*	+6,400 ±1,600 S‡	+4,000 ±1,400 s‡
Lymphocytes (Absolute)*	− 400 ±800	−2,500 ±1,000 s
Neutrophils (Absolute)*	+6,400 ±1,400 S	+6,400 ±1,600 S
Eosinophils*	+ 54 ±25	− 95 ±25 S
Eosiniphils % change	+ 48%	− 54%
Blood Glucose (mg./100 cc.)		
M.D. ±S.E.†	+ 85.7 ±18.8 S	+ 71.7 ±26.5 s
% change	+ 75%	+ 50%

* Mean differences: number of cells/cu. mm. before, and 3½ after, injections (±S.E.).
† Mean differences: mg. glucose/100 cc. blood before, and 3½ hrs. after, injections.
‡ S = P < 0.01; s = P < 0.05.

ascorbic acid failed to prevent the hyperglycemic action of epinephrin, it did succeed in blocking the hematologic signs of the alarm reaction. The leukocyte responses in this experiment confirm similar observations in rats and mice.

Experiment III: This experiment was conducted in order to determine whether the blocking of the hematologic response to epinephrin by ascorbic acid as noted above was due to a masking of the adrenal to the action of endogenously released adrenocorticotrophin. Four guinea pigs were injected intraperitoneally with ascorbic acid (100 mg./100 g.b.w.) and at the same time given single subcutaneous injections of 3.5 mg. A.C.T.H. Blood samples were taken just prior to, and 3½ hours after, the injections.

Results: The data are presented in Table 3 and show that the injection of A.C.T.H. to these vitamin-treated guinea pigs induced a significant lymphopenia, neutrophilia, and an eosinopenia. The blood glucose level was not markedly altered.

Experiment IV: This experiment was conducted on bilaterally adrenalectomized guinea pigs. The animals were adrenalectomized in two stages, the last operation at least one week before this experiment. They

TABLE 3. HEMATOLOGIC RESPONSE TO A.C.T.H. IN ASCORBIC ACID TREATED INTACT GUINEA PIGS

Treatment	A.C.T.H.
No. of animals	4
Leukocytes (Total)*	+6,000 ±1,400 S†
Lymphocytes (Absolute)*	−3,500 ±1,300 s†
Neutrophils (Absolute)*	+9,500 ±4,300
Eosinophils*	−100 ±40 s
Eosinophils % change	− 47%
Blood Glucose (mg./100 cc.)	
M.D. ±S.E.‡	+100 ±13.0
% change	+7.9%

* Mean differences: number of cells/cu. mm. before, and 3½ hrs. after, injections (±S.E.).
† S = P < 0.01; s = P < 0.05.
‡ Mean differences: mg. glucose/100 cc. blood before, and 3½ hrs. after, injections (±S.E.)

were maintained on saline and Rabbit Chow. Five animals received intraperitoneal injections of ascorbic acid (100 mg./100 g.b.w.) and were given at the same time 2.5 mg. Cortisone acetate per 100 g. body weight. Blood for leukocyte counts was taken just prior to, and 3½ hours after, the injections.

Results: The data are presented in Table 4. It is seen that the characteristic hematologic signs are observed, i.e., lymphopenia, neutrophilia, and eosinopenia. Blood glucose determinations were not made.

DISCUSSION

It has been shown in other studies (Dugal, 1949) that ascorbic acid treatment to rats and guinea pigs prolonged their survival times under the stress of cold, and prevented certain signs of an adaptive response, viz, adrenal and kidney hypertrophy. It is well known that treatment of animals under cold with cortical hormone also prolongs their survival time. It was shown subsequently (Bacchus, Toompas, and Heiffer, 1951) that the protective action of the vitamin requires the presence of the intact adrenal. Cortical hormones, on the other hand, exert their protective action even in the absence of the adrenal.

TABLE 4. HEMATOLOGIC EFFECT OF CORTISONE IN ASCORBIC ACID TREATED ADRENALECTOMIZED GUINEA PIGS

Treatment	Cortisone
No. of animals	5
Leukocytes (Total)*	+ 800 ±2,300
Lymphocytes (Absolute)*	−4,700 ±1,700 s†
Neutrophils (Absolute)	+3,700 ±900 S†
Eosinophils*	− 242 ±22 S
Eosinophils % change	− 57%

* Mean differences: number of cells/cu. mm. before, and 3½ hrs. after, injections (±S.E.).
† S = P < 0.01; s = P < 0.05.

Sayers and Sayers (1947) observed that the pretreatment of animals with cortical hormones prevented the depletion of adrenal ascorbic acid when the animals were subsequently exposed to cold, heat, epinephrin, and histamine stressors. Ascorbic acid pretreatment, similarly, prevented the release of cytochemically detectable steroids from the adrenal of the rat injected with epinephrin (Bacchus *et. al.*, 1951). Cortical hormone pretreatment did not prevent the adrenal ascorbic acid depletion in animals given A.C.T.H. (Sayers and Sayers, 1947), nor did ascorbic acid pretreatment prevent the typical leukocytic changes in A.C.T.H. treated animals (Bacchus and Altszuler, 1951; data in the present experiment).

Sayers and Sayers (1947) have shown that the degree of release of adrenocorticotrophin depends on the titer of the cortical steroids in the blood. Cortical steroid pretreatment, therefore, by maintaining the titer of the hormones under the various conditions of stress, prevented the release of pituitary adrenocorticotrophic hormone. It is apparent from the data in the present study that the pituitary-adrenal axis did not respond to epinephrin in the ascorbic acid-treated guinea pigs. This is probably not due to a specific anti-epinephrin action of the vitamin since epinephrin still exhibited its hyperglycemic action in these animals. Furthermore, the prevention of activation of the pituitary-adrenal axis is not due to the inhibition of the actions of A.C.T.H. or cortisone as the data in experiments III and IV indicate. Therefore it becomes necessary to suggest that the characteristic hematologic action of epinephrin was not observed in the ascorbic acid-treated guinea pigs because of a prevention of the release of adrenocorticotrophin by the anterior pituitary. The exact details of this mechanism are not known, but preliminary data indicate that the vitamin may support glucocorticoid hormones in certain of their metabolic functions. It has been observed in preliminary studies that the vitamin is capable of prolonging the hematologic as well as the gluconeogenic actions of cortisone. It is possible that the vitamin aids in maintaining the titer of cortical steroids even under conditions of greater "utilization" of the hormones.

SUMMARY

The action of ascorbic acid treatment to guinea pigs on the blood leukocyte and blood glucose responses to epinephrin, adrenocorticotrophin, and cortisone was investigated. The action of ascorbic acid treatment *per se* on those patterns and on the adrenal cholesterol level was also investigated. The data indicate that ascorbic acid *per se* does not alter the leukocyte and blood glucose levels. The vitamin is capable of altering the leukocyte response to epinephrin, but not to adrenocorticotrophin or cortisone. It was shown that this action of the vitamin is probably not due to a

specific antagonsim to epinephrin. It is suggested that the vitamin probably prevented the release of adrenocorticotrophin by the anterior pituitary. The details of this mechanism are under investigation.

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INFLUENCE OF DIFFERENT DIETARY PROTEIN LEVELS IN STARVATION ON THE ENDOCRINE SYSTEM OF MALE AND FEMALE RATS*

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IT HAS been repeatedly demonstrated that starvation in rats results in diminution in weight and function of the endocrine glands. This has been attributed to impairment of secretory activity of the anterior pituitary (Mulinos and Pomerantz, 1939, 1940, 1941c, Mason and Wolfe, 1930, Werner 1939). On the other hand, differences in the response of the adrenals have been noted in accordance with the severity and duration of the starvation. In general, it has been found that acute starvation leads to adrenal hypertrophy, whereas chronic starvation, in rats, causes adrenal atrophy (Mulinos and Pomerantz, 1940, 1941a, Cameron and Carmichel, 1946).

The present studies were undertaken in order to determine whether some of the effects of starvation are dependent, qualitatively or quantitatively, on the caloric restriction alone or on the level of dietary protein.

MATERIALS AND METHODS

Adult male and female rats of the Wistar strain, bred by the Albino Farms, Redbank, New Jersey were used. The animals were kept in individual wire cages in an air-conditioned room at a temperature of 72° F. The water and food intake and the body weight of each animal were determined daily.

All animals were given diet A (Table 1) for an observation period of 6 days, and their daily food consumption was recorded. Subsequently the rats were divided into 3 groups. Group I (Control) continued on the same diet, eating food ad libitum. Group II (starvation) received one-fourth of the amount of Diet A consumed in the pre-experimental period. These rats then received $\frac{1}{4}$ of the calories and $\frac{1}{4}$ of the protein consumed by the controls. Group III (starvation) received the same amount of food as

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Group II, but was given Diet B. Diet B was isocaloric with Diet A, so that the two starvation groups had the same caloric intake. However, the protein content of Diet B was four times that of Diet A; consequently, starvation Group II received the same amount of protein as did the fully fed controls. The composition of the diets used is recorded in Table 1. All animals were sacrificed after 15 days on their respective diets. In order to insure a constant daily intake of iodine, all animals received iodine-free (triple distilled) water and daily intraperitoneal injections of two micrograms of iodine as potassium iodide dissolved in 0.5 ml. of triple-distilled water. At the conclusion of the experiment, thyroid function was determined by measuring the uptake of radioactive iodine. Four microcuries of I^{131}* were administered intraperitoneally as sodium iodide

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Dietary constituents	Diet A (100 gm.)	Diet B (100 gm.)
Dextrose[1]	72	24
Casein[2]	16	64
Corn Oil[3]	8	8
Salt Mixture[4]	4	4

To each 100 gm. of the above diets were added the following synthetic vitamins: Riboflavin, 0.3 mgm.; Thiamine Hydrochloride, 0.2 mgm.; Nicotinic Acid, 2.5 mgm.; Calcium Pantothenate, 4.0 mgm.; Pyridoxine, 0.5 mgm.; Inositol, 10.0 mgm.; Choline Chloride, 100.0 mgm.; Methyl Naphtoquinone, 1.0 mgm. Each rat also received three times weekly, cod liver oil, 2 drops and alpha-tocopherol, 1 mgm.

[1] Dextrose anhidrous, Merck and Co. Inc.
[2] Vitamin Test Casein, G.B.I.
[3] Mazola.
[4] Salt Mixture No. 2—U.S.P. XII.

without carrier. The rats were killed four hours after injection. The wet weights of the dissected pituitaries, adrenals, thyroids, kidneys, gonads and seminal vesicles or uteri were determined on a Roller-Smith torsion balance. After the weight of the thyroid was determined, it was immersed in 3 ml. of a 2 per cent solution of sodium hydroxide. Aliquots (0.2 ml.) were placed on copper planchettes, dried, and the radioactivity determined by means of a Geiger-Müller counter.

One group of male rats was castrated; after 3 weeks the animals were divided into two groups and received the two starvation diets respectively for 23 days. Testosterone propionate, 0.5 mg. daily, was administered on days 20, 21, and 22. Animals were sacrificed on day 23 and weights of the seminal vesicles and prostate were determined.

RESULTS

Body Weight: The control animals gained weight during the experimental period, the males showing a mean gain of 22 per cent and the females of 6 per cent. Both male and female rats on starvation diets lost weight, the mean weight loss varying between 30 and 34 per cent. There was no difference between groups receiving high and low protein levels (Tables 2, 3).

* Radioactive Iodine (I-131) was supplied by the Atomic Energy Commission, Oak Ridge, Tennessee.

TABLE 2. BODY WEIGHT AND ORGAN WEIGHT (WITH STANDARD ERROR) OF RATS FED ½ OF INTAKE OF CONTROLS FOR 16 DAYS. DIET A LOW PROTEIN, DIET B HIGH PROTEIN (SEE TABLE 1)

Males	Body weight initial	(gm.) Final	Thyroid mgm.	Adrenal mgm.	Pituitary mgm.	Kidney gm.	Testes gm.	Seminal vesicles gm.
Control group Diet A ad lib. (12 rats)	181 ±2.19	224 ±4.79	12.26 ±0.42	33.65 ±1.94	7.9 ±0.29	1.26 ±0.08	2.40 ±0.13	1.12 ±0.10
Starvation group I Diet A ½ (11 rats)	187 ±4.39	122 ±5.83	7.67 ±0.57	28.38 ±1.32	5.36 ±0.22	0.76 ±0.05	1.47 ±0.14	0.18 ±0.02
Starvation group II Diet B ½ (11 rats)	191 ±4.51	127 ±5.41	7.74 ±0.41	31.06 ±1.34	4.96 ±0.13	1.02 ±0.04	1.72 ±0.14	0.29 ±0.04
Females							Ovaries mgm.	Uterus mgm.
Control group Diet A ad lib. (11 rats)	173 ±4.57	184 ±5.03	12.25 ±0.59	39.45 ±2.36	10.90 ±0.36	1.16 ±0.04	120.90 ±7.99	390.90 ±18.68
Starvation group I Diet A ½ (9 rats)	173 ±5.19	117 ±3.08	3.75 ±0.44	33.11 ±1.71	4.43 ±0.33	0.78 ±0.03	40.88 ±1.70	122.22 ± 5.81
Starvation group II Diet B ½ (8 rats)	168 ±4.91	114 ±5.19	3.52 ±0.26	32.50 ±1.97	4.5 ±0.26	0.88 ±0.01	45.25 ±1.72	180.00 ±13

Endocrine Glands: (Tables 2, 3): The pituitary weights decreased in all starvation groups.

This weight loss was much greater in the females, the pituitary weights of starved females becoming equal to those of the starved males, whereas

TABLE 3. RATS FED ½ INTAKE OF CONTROLS FOR 16 DAYS. DIET A LOW PROTEIN. DIET B HIGH PROTEIN. ORGAN WEIGHTS PER 100 GM. BODY WEIGHT

Males	No. of animals	Body weight gain or loss	Pituitary mg.	Thyroid mg.	Adrenal mg.	Testes gm.	Seminal vesicles gm.	Kidney gm.
Control group Diet A ad lib.	12	+22%	3.51	5.45	14.25	0.99 ±0.65*	0.49 ±0.04*	0.57 ±0.02*
Starvation group I ½ Diet A	11	−35%	4.53	6.28	23.50	1.15 ±0.09*	0.14 ±0.01*	0.62 ±0.03*
Starvation group II ½ Diet B	11	−34%	3.96	6.17	24.70	1.40 ±0.10*	0.23 ±0.01*	0.81 ±0.02*
Significance of Difference of Starvation A and B "P"						<0.1	<0.01	<0.01
Females						Ovaries mg.	Uterus mg.	
Control group Diet A ad lib.	11	+ 6%	5.92	6.59	21.49	64.47 ±4.42*	221.45 ±10.55*	0.62 ±0.02*
Starvation group I ½ Diet A	9	−33%	3.83	3.20	28.39	35.03 ±1.89*	105.53 ±7.08*	0.67 ±0.03*
Starvation group II ½ Diet B	8	−33	4.16	3.08	28.60	40.80 ±2.74*	151.11 ±9.48*	0.77 ±0.03*
Significance of Difference of Starvation A and B "P"						<0.1	>0.01	<0.05

* Standard error.

in the ad lib fed controls the well known greater weight of the female pituitaries was evident (Table 2). Calculation of the pituitary weight per unit of body weight indicates that the weight of the pituitary of the female rats on both high and low protein starvation diets was reduced relatively more than the body weight. The difference between the pituitary weights (both absolute and relative to body weight) in the two starvation groups was not significant statistically. The weight loss of the pituitary of the starved male rats was proportional to the body weight loss in the high protein starvation group. In the low protein starvation group there was a "relative" increase in pituitary weight (Table 3).

The weight of the thyroid glands of all animals was decreased, but the diminution in weight was more marked in females than in males. In the latter, the weight loss was proportional to the body weight loss, whereas in the former the weight loss of the thyroids exceed that of the whole body. In neither sex was there any difference between starvation groups fed low and high protein diets.

There was no change in the absolute weights of the adrenals in any of the starvation groups, but if one computes the adrenal weight in relation to the final body weight there appears to be an increase in these groups. There was no difference in adrenal weight in starved animals receiving low and high protein diets.

The testis weights of the starved animals was decreased, approximately in proportion to the body weight loss. The weight loss of the ovaries, however, was considerably greater than that of the total body. The weight loss of the ovaries and testes appeared to be less marked in the starved animals on high, than on low protein intakes, but the differences were not significant statistically.

Accessories: Seminal vesicles and uterus were selected as representing structures influenced by the steroid secretion of testes and ovaries respectively. The weights of both the seminal vesicles and the uterus were decreased in starvation, in both instances relatively more than the total body weight. Both the seminal vesicles and the uterus lost significantly less weight in starved animals on the high protein intake than in those on the low protein diet.

Data presented in Table 4 indicate that the response of the seminal vesicle and prostate of castrate rats to testosterone propionate (total dose 1.5 mg.) was identical in starved animals on the high and low protein intakes.

Kidney: The weight loss of the kidneys was approximately proportional to the body weight loss in the animals receiving the low protein diet, but was less than the body weight loss in those on the high protein diet.

TABLE 4. CASTRATE MALE RATS ON LOW PROTEIN (A) AND HIGH PROTEIN (B) STARVATION DIET FOR 23 DAYS. 0.05 MG. TESTOSTERONE PROPIONATE INJECTED S.C. ON DAYS 20, 21, 22. WEIGHT OF SEMINAL VESICLES AND PROSTATE

	Body weight Loss	Seminal Vesicles Mgm.	Prostate Mgm.
Starvation Group A (7 rats)	46%	116 ± 10.2*	83 ± 6.02*
Starvation Group B (high protein) (10 days)	42%	108 ± 6.7*	80 ± 4.51*

* Standard error.

Function of the Thyroid: The function of the thyroid gland under the conditions of the two types of starvation was studied by means of uptake of I^{131} (Table 5). The uptake in the entire thyroid gland was decreased in male and female animals at both levels of protein intake. In view of the fact that the thyroid weight was decreased (Table 2), this decreased uptake might be the result of decreased thyroid mass. However, calculation of the uptake per mg. of thyroid tissue (counts/sec/mg) reveals that thyroid function is actually decreased. The differences are statistically significant for males starved at high and low dietary protein levels, and for females on the low protein diet; for the starved females on the high protein diet the difference is not significant statistically. The lowering of thyroid function is of the same degree in groups fed low protein and high protein starvation diets.

Discussion: The effects of starvation upon the endocrine glands has

TABLE 5. UPTAKE OF TRACER DOSE (4μc) OF I-131 4 HOURS AFTER I.P. INJECTION BY THYROID OF CONTROL AND STARVED RATS

Group	No. of rats	Weight mg.	Counts/sec/mg. Mean and standard error	Counts/sec/gland mean
Males				
Control				
Diet A ad lib.	12	12.3	40.5 ±4.89	496.08
Diet A ¼	11	7.7	27.7* ±1.67	207.09
Diet B ¼	11	7.7	24.5† ±2.13	195.06
Females				
Control				
Diet A ad lib.	11	12.3	49.6 ±5.15	591.4
Diet A ¼	9	3.8	34.4* ±3.24	136.01
Diet B ¼	8	3.5	42.2‡ ±5.46	136.04

* Difference from control: P = <0.05.
† Difference from control: P = <0.01.
‡ Difference from control: P = <0.4.

been the subject of many investigations. Briefly stated, the problem is whether starvation causes a diminution in size and/or function of the endocrine glands proportional to the loss of body weight or whether specific changes occur. In the studies reported here the influence of different dietary protein levels was investigated in order to determine whether the protein level might be a limiting factor in changes resulting from starvation. The two groups of starved animals had the same caloric intake, 25% of that of the controls. In one group (low protein) the protein intake was also 25% of that of the controls, whereas the diet of the second group (high protein) was adjusted so as to make their protein intake identical with that of the controls, and four times that of the "low protein" group.

The observation that in starvation the pituitary weights in male rats decreased proportionally to the body weight confirms previous observations of Jackson (1917) and of Mulinos and Pomerantz (1940). In our female rats the fall in pituitary weight was relatively greater than that in body weight. The level of protein in the hypocaloric diet did not influence the weight loss. Friedman and Friedman (1940) failed to detect any difference in the gonadotrophin content of the pituitaries of rabbits fed a full diet varying in protein content.

The adrenal glands in our experiment did not participate in the general weight loss, the weights of the adrenals in starvation being identical with those of the controls. This was true at both high and low dietary protein levels. Relative to the final body weight of the starved animals, the adrenals were larger than in the normal controls. This "relative hypertrophy" is similar to that observed in acute starvation (D'Angelo *et al.* 1948). On the other hand, Mulinos and Pomerantz (1940) reported absolute increase in adrenal weight in completely starved rats. Atrophy of the adrenals has been reported in prolonged chronic inanition (Jackson 1917, Mulinos and Pomerantz 1941a, Cameron and Carmichel 1946). The differences between these findings and ours may well be due to differences in severity and duration of inanition. Our animals lost 30–34 per cent of the body weight within 15 days, whereas in the experiments of Mulinos and Pomerantz a weight loss of 23 per cent occurred in 106 days.

Thyroid: Atrophy of the thyroid gland in starvation has been described by Mulinos and Pomerantz (1940). In our experiments the weight of the thyroids of all starved animals decreased, regardless of the protein content of the hypocaloric diets. In male rats, the weight loss was approximately proportional to the loss of body weight, whereas, in females, the decrease in thyroid weight was relatively greater than that in body weight. These findings differ from those of Meites and Reed (1949), who claimed that both in total and in partial starvation the thyroid weight decreases only in proportion to the decrease in total body weight. It appears to us that undue em-

phasis has been placed on the fact that in some instances the relative weight of the thyroid gland (Mg/100 gm body weight) is not decreased in starvation. The weight loss of the entire animal is accounted for to a large extent by loss of body fat. Only to the extent to which weight loss is due to catabolism of cellular protein, could one expect the thyroid to participate in the general pattern. In view of the large contribution of fat loss from the carcass to the total weight loss of the starved animal, it would probably be more nearly correct to calculate the relative weight loss of the thyroid in relation to initial rather than final body weight. On this basis, the relative thyroid weights of both female and male starved rats decreased, regardless of the protein levels fed, the decrease being greater in the female rats.

There is general agreement that thyroid function is diminished in starvation. This, as well as the decrease in size discussed above, has been attributed to diminished secretion of thyrotrophic hormone by the anterior pituitary. This has actually been demonstrated by assay of TSH levels in the serum of acutely starved rats (D'Angelo 1951). In the present study, thyroid function, gauged by the uptake of a tracer does of I^{131}, was found to be diminished. This diminution was due not only to the decrease in thyroid mass (weight), but also to a decrease in function of the remaining thyroid tissue. No differences were observed between the animals on the two different levels of dietary protein.

These findings are in disagreement with those of Meites and Wolterink (1950), who found that both thyroid weight and function decreased in proportion to the body weight loss; uptake of I^{131} per mg. of thyroid tissue, or per gram of body weight, was not changed in their starved animals. No definite explanation can be given for this descrepancy, but certain differences in technique may be important. The iodine intake, variations in which would influence uptake of I^{131} by the thyroid, was rigidly controlled in our experiements but not in those of Meites and Wolterink. Furthermore, the technique employed for measuring radio activity differed, inasmuch as we used small aliquots of glands extracted and macerated in KOH, whereas Meites and Wolterink dried the entire gland on a planchet.

Genital System: The testes of the starved animals were atrophic, in agreement with previous observations (Mason and Wolfe 1930, Mulinos and Pomerantz 1940). This atrophy is probably due to diminished gonadotrophic stimulation inasmuch as it can be corrected by treatment with gonadotrophic hormones during continued hypoalimentation (Mulinos and Pomerantz 1941b,c, Vollmer 1943). In our experiements, the decrease in testis weight was greater in animals receiving the low protein diet, but the difference between the two starvation groups is not statistically significant ("P" <0.1). The atrophic testes secrete subnormal amounts of andro-

gens, as evidenced by the atrophy of the seminal vesicles, also described by Mulinos and Pomerantz (1940). The atrophy of the seminal vesicles in our experiments was significantly greater in the animals on the low protein diet ("P" <0.01). This would suggest either that during starvation the testes of animals receiving relatively high levels of protein produce more androgen than those of animals on a low protein intake, or that the level of dietary protein is a limiting factor in the response of the seminal vesicle to endogenous androgens. However, the latter explanation is not supported by the observation that the seminal vesicle response to exogenous testosterone was identical in castrate rats receiving low and high protein starvation diets. This makes it appear likely that the testis in starved rats secretes more testosterone if the hypocaloric diet contains large amounts of protein.

Atrophy of the ovaries was present in all starved animals, confirming the observations of Marrian and Parks (1929), Mulinos *et al.* (1939), Werner (1939) and Stephens and Allen (1941). As in the case of the testes, the ovarian atrophy was more severe in animals on low protein intake, but the difference between the latter and those on the high protein diet was not significant statistically. The diminished estrogen secretion was evidenced by decreased uterine weight. The latter was significantly lower in the low protein starvation group than in the high protein starvation group ("P" <0.01). The same possibilities mentioned in discussing the atrophy of the seminal vesicles apply here. Either the ovary of starved rats on the high protein intake secretes more estrogen, or the protein levels in the diet influence the response of the uterus. Target organ sensitivity was not tested in the female, but the findings in the male make it appear probable that hormone secretion by the gonad during starvation is higher on high than on low protein diets.

Kidney: The loss of kidney weight in starvation has been shown to be relatively less than that of body weight (Mulinos and Pomerantz 1941c, Quimby 1948). The loss of kidney weight in our experiments was significantly greater in the animals on the low protein diet. The influence of the protein content of calorically adequate diets on kidney weight has been demonstrated previously by Reid (1944) and Leathem (1945). The increase in kidney weight in animals receiving high protein diets could not be demonstrated in hypophysectomized rats, suggesting that pituitary function either is a prerequisite, or mediates this effect of high protein diets. If the same mechanism can be assumed to operate in the protection against kidney weight loss in our starved rats, the pituitary mechanism would appear to be still effective in the presence of decreased pituitary function incident to starvation.

Sex Difference: Although the body weight loss during starvation did not

differ in the two sexes, the pituitary weight loss was much greater in the females than in the males. Only in the females did the pituitary weight decrease relatively more than the body weight. The data on thyroid and gonadal weights suggest that secretion of thyrotrophic and gonadotrophic hormones was similarly decreased more markedly in the female, inasmuch as the absolute weight loss of the thyroid was 37 per cent in the male, and 69 per cent in the female, that of the testes 38 per cent and of the ovaries 66 per cent. The relative weight loss of the thyroid and gonads exceeded that of the body in the females, but not in the males. It would appear that the female pituitary is much more sensitive to starvation than that of the male.

SUMMARY

Starvation experiments were conducted for 16 days on female and male rats, the diets containing 25 per cent of the calories consumed by the normal controls. Two types of starvation diets were employed, one being the same as that given to the control animals; this "low-protein" starvation group therefore received 25 per cent of the quantity of protein given to the controls. The second, "high-protein" starvation group received a diet isocaloric with that of the low-protein group, but containing four times as much protein. Their protein intake was therefore identical with that of the controls.

Body weight loss was identical in the two starvation groups.

The weight loss of the pituitaries was more marked in the females than, in the males. In the latter it was proportional to the loss of body weight, whereas it exceeded the body weight loss in the females. The different levels of dietary protein did not influence the degree of pituitary weight loss.

The thyroid weights in the males decreased in proportion to the loss of body weight; in the females the relative weight loss of the thyroid exceeded that of the body. Thyroid function, as determined by uptake of I^{131} per mg. of thyroid tissue was decreased. Neither the weight loss nor the decreased function of the thyroid was influenced by the level of dietary protein.

The weight of the adrenal glands of all starved animals was identical with that of the controls, indicating that the adrenals did not participate in the general weight loss. Thus, there was "relative hypertrophy," such as is observed in acute, complete starvation, and not the atrophy of chronic inanition.

The weight of the testes and ovaries decreased in starvation. The weight loss was less severe in animals on the high protein intake, but the differences in gonadal weight in the two starvation groups were not significant statistically.

In contradistinction to the failure of the different protein levels to in-

fluence the weight loss of pituitary thyroid and gonads, the decrease of thyroid function, and the "relative weight gain" of the adrenals in starvation, there were significant differences between the low and the high protein starvation groups in other respects. The weight loss of the seminal vesicles and of the uterus, chosen as representative target organs of androgens and estrogens, respectively, was significantly greater in the low protein than in the high protein starvation group. The response of the seminal vesicles of starved castrate males to exogenous testosterone propionate was not influenced by the dietary protein level. This suggests that the testes of rats receiving the high-protein starvation diet secrete more testosterone than do those on the low-protein starvation diet.

The weight loss of the kidneys was significantly greater in the low than in the high protein starvation group.

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INFLUENCE OF THE ADENOHYPOPHYSIS ON THE FROG WATER-BALANCE RESPONSE[1]

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IN THE intact frog the injection of posterior pituitary extract causes a marked increase in the rate of water uptake. This water-balance response is the result of an increase in the permeability of the skin to water and is produced by the oxytocic fraction. Adenohypophyseal function appears to be necessary for a normal water-balance response. Jones and Steggerda (1935) reported a complete loss of the response in frogs hypophysectomized more than one week. Chen *et al.* (1943) noted that the response declines to 25 per cent of normal ten days after removal of the adenohypophysis but does not disappear completely.

This paper concerns the changes in water balance that occur in the frog following hypophysectomy. Alterations in permeability of the skin as well as in the response to pitocin have been observed. The response has also been restored to normal by means of appropriate hormone replacement in the hypophysectomized frog.

MATERIALS AND METHODS

Male *Rana pipiens* averaging about 30 gm. were used. The frogs were kept in 100 ml. of tap water in individual beakers at room temperature for at least 16 hours previous to, and during, all experiments. They were not fed.

Total hypophysectomy was performed under ether anesthesia. The gland was approached through the roof of the mouth and removed by suction through a small trephined hole in the parasphenoid bone. Operated and control frogs were kept in a cold room at 9° C for at least five days before use in an experiment.

Cloacal ligation was accomplished by a single cotton purse-string tie around the external aperture, under light ether anesthesia. Before ligation urine was expressed from the bladder by abdominal pressure.

All materials were injected in solution into the dorsal lymph sac. Controls received injections of the solvents used in amounts equivalent to those in the corresponding experimental injections.

The water-balance response was determined by the method of Sawyer *et al.* (1950) over a three hour period after the injection of one unit of pitocin/100 gm. body weight. Weight gain is recorded as per cent of the original body weight.

Numerical results are expressed as arithmetic means plus or minus the standard errors of the means. The t test is used as a measure of statistical significance. P values are from Fisher's (1948) table of t for small samples.

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RESULTS

Effect of hypophysectomy on the water-balance response

The average water-balance response of 136 control frogs was 9.1 ± 0.9 per cent after the injection of one unit of pitocin/100 gm. When tested five days to two weeks after operation hypophysectomized frogs showed a response of 2.5 ± 0.6 per cent, or 27.5 per cent of the control value. Frogs used five days after hypophysectomy showed a response 31.3 per cent of normal, and at two weeks after operation, 25.2 per cent. In no experiment did the response of hypophysectomized frogs average less than 20 per cent of the control value. These results are in agreement with those of Chen *et al.* (1943), who found that the response declined to 25 per cent of normal ten days after hypophysectomy, rather than the results of Jones and Steggerda (1935), who found a complete loss of response one week after operation.

Effect of hypophysectomy on the permeability of frog skin and the response to pitocin

The permeability of the frog skin to water and its response to pitocin were determined by a simple *in vivo* method. The cloacal apertures of a

TABLE 1. EFFECT OF HYPOPHYSECTOMY ON THE PERMEABILITY OF FROG SKIN TO WATER AND ON THE WATER-BALANCE RESPONSE

	No.	Rate of entry of water, $mm^3/cm^2/hour$	
		Control period	Pitocin period
Normal frogs	10	5.2 ± 0.4	27.3 ± 1.5
Hypophysectomized frogs	18	8.0 ± 0.8	11.3 ± 1.0

group of normal and hypophysectomized frogs, in which the water-balance response had declined, were ligated. The frogs were weighed and injected with 1 ml/100 gm. of 0.5 per cent chloretone. Three hours later the frogs were reweighed and given one unit/100 gm. of pitocin. Three hours later the frogs were again weighed. The rate of inflow of water through the skin was calculated from the weight gain using Adolph's (1931) formula for body surface, $BS = 8 \times (BW)^{2/3}$.

Hypophysectomy produces a slight increase in the permeability of the skin to water (table 1). The difference between normal and hypophysectomized frogs during the control period is statistically significant ($P < 0.01$). It thus appears that the adenohypophysis is necessary for the maintenance of normal skin permeability. Adenohypophyseal hormones are also necessary for the normal action of pitocin as shown by the dif-

ference ($P < 0.001$) in the rate of water uptake between normal and hypophysectomized frogs after the injection of pitocin.

The reliability of this simple method of determining the rate of entry of water through the frog skin seems good. During the control period in normal frogs the rate was 5.2 ± 0.4 mm³/cm²/hour. Hevesy, Hofer and Krogh (1935) found water uptake to be 5 mg/cm²/hour in the intact frog by using heavy water. With isolated frog skins Sawyer (1951) found a corresponding value of 4.73 ± 0.74 mm³/cm²/hour.

Effect of hormone replacement on the water-balance response of hypophysectomized frogs

The anterior lobe of the pituitary produces a number of hormones which may act directly on non-endocrine tissues or may stimulate other glands

TABLE 2. EFFECT OF THYROXIN ON THE WATER-BALANCE RESPONSE OF NORMAL AND HYPOPHYSECTOMIZED FROGS

Group	No.	Daily dose mg/100 gm.	Water-balance response, per cent gain in weight	
			First day	Fifth day
Normal	8	0.0	9.6±0.9	9.1±1.1
Normal	8	0.1	8.4±1.0	10.6±1.0
Hypophysectomized	8	0.0	2.8±1.0	3.0±1.1
Hypophysectomized	8	0.1	3.4±1.0	5.4±0.8
Normal	8	0.0	14.9±1.1	15.1±1.0
Normal	8	1.0	11.6±0.8	15.5±1.1
Hypophysectomized	8	0.0	5.4±0.9	4.2±0.6
Hypophysectomized	8	1.0	2.7±0.6	5.1±0.6

to produce the hormones which act on the ultimate target organs. Many hormones may therefore be concerned in the action of the adenohypophysis on the frog water-balance response. Several have been tested to determine whether they are capable of returning the decreased water-balance response of hypophysectomized frogs to normal.

In each experiment four groups of frogs were used: untreated normal, hormone treated normal, untreated hypophysectomized, and hormone treated hypophysectomized. The water-balance response of each frog was determined on the first day of the experiment and again on the fifth day. Hormones were injected daily for four days.

Thyroxin. In two separate experiments crystalline thyroxin was administered in doses of 0.1 and 1.0 mg/100 gm/day. Thyroxin increases the water-balance response of both normal and hypophysectomized frogs to some extent (table 2). Both doses are equally effective but the response does not approach normal.

ACTH. Adrenocorticotropic hormone (ACTH) was injected in doses of 1.0 and 1.5 mg/day (1 mg. = one La-1-A unit) in two experiments. ACTH increases the water-balance response of hypophysectomized frogs to about three-fourths of normal (table 3). This response could not be due to contamination of the ACTH with posterior lobe hormone. The last injection of ACTH was given 24 hours before the water-balance response was tested. The effect of posterior lobe extracts does not last over about three hours at this temperature. The ACTH preparation was tested, however, for its water-balance effect, and, at these doses, was not found to produce a significant weight gain.

TABLE 3. EFFECT OF ACTH ON THE WATER-BALANCE RESPONSE OF NORMAL AND HYPOPHYSECTOMIZED FROGS

Group	No.	Daily dose mg./100 gm.	Water-balance response, per cent gain in weight	
			First day	Fifth day
Normal	15	0.0	6.7±0.7	8.0±0.6
Normal	15	1.0	7.8±0.7	9.5±0.8
Hypophysectomized	15	0.0	2.2±0.9	2.7±0.7
Hypophysectomized	15	1.0	2.2±0.8	6.2±0.8
Normal	8	0.0	7.6±0.6	7.4±0.8
Normal	8	1.5	8.0±0.7	9.5±0.7
Hypophysectomized	8	0.0	1.9±0.9	2.2±0.9
Hypophysectomized	8	1.5	2.3±0.7	6.5±0.7

TABLE 4. EFFECT OF THYROXIN PLUS ACTH ON THE WATER-BALANCE RESPONSE OF NORMAL AND HYPOPHYSECTOMIZED FROGS

Group	No.	Daily dose mg./100 gm.		Water-balance response, per cent gain in weight	
		Thyroxin	ACTH	First day	Fifth day
Normal	6	0.0	0.0	8.9±1.0	8.9±1.1
Normal	6	0.1	1.0	7.9±0.9	9.6±1.0
Hypophysectomized	8	0.0	0.0	2.4±0.8	2.3±0.7
Hypophysectomized	10	0.1	1.0	2.7±0.7	8.5±0.9

Adrenal cortical preparations. In separate experiments cortical extract (Eschatin, Parke, Davis), 1 ml/100 gm/day; desoxycorticosterone glucoside (DCG), 1.0 and 2.5 mg/100 gm/day; DCG plus cortical extract, 1.0 mg. and 1 ml/100 gm/day; cortisone acetate, 1.0 mg/100 gm/day, were each given for four days. In no case did such treatment cause a significant change in the water-balance response of hypophysectomized or control frogs.

ACTH plus thyroxin. ACTH, 1.0 mg/100 gm/day, plus thyroxin, 0.1 mg/100 gm/day, were given for four days. This treatment restores the response of hypophysectomized frogs to normal (table 4).

DISCUSSION

Water ordinarily passes into the frog at a slow rate; the skin acts as a barrier to the free osmotic inflow (Adolph, 1933). The integrity of this barrier may be damaged by brain injuries (Adolph, 1931, 1934), anoxia, mechanical trauma, or poisoning (Maxwell, 1913; Adolph, 1925). From the experiments presented above it appears that certain hormones are necessary for the normal resistance of the skin to the inflow of water. Thyroxin, and presumably certain adrenal hormones, influence the mechanism by which the relative impermeability of the skin is maintained. It remains to be elucidated whether these hormones are related to the impermeability in a general way, acting to maintain the over-all integrity of the cells of the skin, or are concerned specifically in the action of the forces within the skin which oppose the inflow of water.

The water-balance principle of posterior pituitary extract acts to increase the rate of inflow of water through the frog skin. This is accomplished by an increase in the permeability to water, not by active water transport (Sawyer, 1951). The above experiments show that thyroxin, and probably adrenal cortical hormones, are necessary if pitocin is to exert its full effect on the permeability of frog skin. The absence of adenohypophyseal hormones increases the permeability but decreases the response to pitocin. It would appear from this that these hormones have two distinct actions on the frog skin. First, they are involved in the maintenance of the normal relative impermeability of the skin to water. Second, they influence the specific mechanism by which pitocin brings about an increase in permeability.

The nature of the action of thyroxin on the frog skin remains obscure. Heller (1930) reported that thyroxin increases the water-balance response by frogs in the winter, but not in the summer. It has also been shown (Houssay, 1949; Adams, 1933) that hypophysectomy of various amphibians results in an increase in the thickness of the keratinized layer of the skin, which can be reversed by the administration of thyroid hormone or adenohypophyseal extracts. Although hypophysectomy alters the color and texture of frog skin histological examination of skin from normal and hypophysectomized frogs failed to demonstrate any evidence of hyperkeratinization. Thyroxin did not alter the histological structure of the skin of hypophysectomized frogs.

ACTH was found to be very active in increasing the water-balance response of hypophysectomized frogs. Cortical extract, cortisone, DCG, and combinations of these, however, were all inactive. This could be due either to the fact that these are mammalian hormones, or to ACTH acting directly upon the skin. ACTH may evoke the secretion of hormones by the

frog adrenal that differ both functionally and structurally from the substances tested.

Assuming that adrenal hormones are involved in the permeability to water and the response to pitocin, the means by which they exert their action is not clear. It has been shown (Adolph, 1925) that the maintenance of the relative impermeability of the frog skin to water is correlated with a high potential difference across the skin. Williams and Angerer (1950) have shown that adrenalectomy causes a decrease in the electrical potential across frog skin. Adrenalectomy also increases the rate of water uptake by the frog (Angerer and Angerer, 1949). In this paper we have demonstrated that permeability increases after hypophysectomy. It would seem a reasonable hypothesis, therefore, that the increased permeability is at least partly due to a failure of normal production of adrenal hormones, consequent to the loss of the stimulus of pituitary ACTH. No evidence is available, however, to account for the necessity of the adrenal hormones in the action of pitocin on the frog skin. Since nothing is known of the means by which pitocin increases the permeability of frog skin it is impossible to speculate profitably as to how the adrenal hormones could be involved in this mechanism.

SUMMARY

Hypophysectomy results in a decrease in the frog water-balance response to pitocin to about one-fourth to one-third of normal five days to two weeks after operation.

The skin of hypophysectomized frogs is somewhat more permeable to water than the skin of normal frogs. The further increase in permeability of the skin produced by pitocin is much less after hypophysectomy.

Thyroxin increases the water-balance response of hypophysectomized frogs to a slight extent. ACTH increases the response to three-fourths of normal. Thyroxin plus ACTH increases the response to normal levels. However, mammalian adrenal cortical extract, DCG, cortisone, and combinations of these have no effect.

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EFFECT OF CORTISONE TREATMENT FOR 28 DAYS ON RADIO-IODINE METABOLISM IN NORMAL RATS AND ADRENALECTOMIZED RATS MAINTAINED WITH DESOXYCORTICOSTERONE[1]

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CLINICAL studies have suggested that cortisone therapy may cause a decrease in thyroid function (Thorn and Forsham, 1949; Reiss *et al.*, 1949; Hill *et al.*, 1950; Statland *et al*, 1950; Beierwaltes *et al.*, 1950; Wolfson *et al.*, 1950; Frederickson, 1951). Other workers believe that such a depression may occur under various conditions of stress (Williams *et al.*, 1949; Korpassy *et al.*, 1950; Paschkis *et al.*, 1950; Van Middlesworth Lester, 1951; Bogorah and Timiras, 1951). The results of animal experiments have been variable. It seems that several factors may influence the findings, and they will be considered in the following study.

MATERIAL AND METHODS

The animals used in this experiment were adult male rats of the Sprague-Dawley strain, selected for uniformity and with a weight range of 270 to 315 grams. The study was carried out on a total of 86 rats. They received a Rockland Rat Diet which contained 1.2 milligrams of iodine per 100 grams, and tap water. Vitamin A (200 I.U.) and Vitamin D (100 I.U.) were given orally once a week. The animals had free access to food.

Three experiments were done:

Group A contained a total of 28 rats and *Group B* 44 rats. Table 1 shows how they were divided for the experiment. Each adrenalectomized animal was maintained by 15 mg. of desoxycorticosterone acetate (DCA) in the form of pellets.* For the first two days following operation, the animals received by mouth 5% glucose in 0.9% saline, after which they were kept on the same diet as the other rats. The animals treated with cortisone acetate,** received 4 mg. subcutaneously every day for 4 weeks. The cortisone

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acetate was suspended in 0.9% saline and the daily dosage was administered in 0.5 cc. of solution.

Group C contained 14 normal rats. They were selected to weigh approximately the final weight of the treated animals. These controls were used immediately for radioiodine study.

The rats were weighed twice a week on a platform balance. At different times daily food intake was weighed in each group. After treatment with cortisone had been given for 4 weeks, each rat received 4 microcuries of I^{131}*** intraperitoneally. The dose of I^{131} was contained in about 0.5 cc. of 0.9% saline and was measured with a tuberculin syringe. Just after injection each animal was placed in a metabolic cage and all urine

TABLE 1. EXPERIMENTAL GROUPS. EFFECT OF CORTISONE THERAPY ON BODY WEIGHT AND ON THE WEIGHT OF THYROID, ADRENAL GLANDS AND KIDNEYS (MEAN VALUES ± STANDARD DEVIATION)

Experimental groups		No. of animals	Body wt. (gm.)		Thyroid wt. (mg.)		Adrenal glands wt. (mg.)		Kidneys wt. (gm.)	
			Before Treatment	After Treatment	Av.	Av. per 100 g. B.W.	Av.	Av. per 100 g. B.W.	Av.	Av. per 100 g. B.W.
Group A	Normal	12	271.5 ±7.75	330.0 ±18.1	19.37 ±0.97	5.90	22.37 ±1.99	6.94	2.25 ±0.10	0.682
	Normal +Cortisone	16	270.0 ±8.25	226.0 ±15.3	13.73 ±0.98	6.08	9.02 ±1.17	3.99	2.075 ±0.13	0.92
Group B	Normal	8	315.0 ± 8.3	358.0 ±14.0	18.83 ±0.57	5.23	22.85 ±1.24	6.35	2.21 ±0.13	0.617
	Normal +Cortisone	8	310.0 ±13.5	257.0 ±26.0	13.55 ±1.07	5.28	9.3 ±0.65	3.62	2.17 ±0.10	0.844
	Adrenal X +DCA	12	320.0 ±12.0	367.5 ±18.0	18.80 ±0.89	5.12	—	—	2.62 ±0.22	0.713
	Adrenal X +DCA +Cortisone	16	321.8 ±18.0	255.0 ±33.5	14.48 ±1.00	5.68	—	—	2.56 ±0.30	1.005
Group C	Normal	14	—	262.5 ±17.5	16.6	6.32	—	—	—	—

was collected until they were sacrificed. From one to three rats were sacrificed at 1, 2, 4, 6, 12, 24, 48 and 96 hours after injection of I^{131}. At the time of sacrifice the animal was anesthetized with ether. The abdomen was opened, aorta and vena cava severed, and the blood collected in a test tube. The blood was immediately centrifuged and the serum separated.

Studies on the thyroid: Each thyroid was dissected free from fat and connective tissue, and was weighed immediately on a Roller-Smith torsion balance. The adrenal glands and kidneys were weighed similarly. The thyroid was transferred into a small Erlenmeyer flask containing 25 cc. of 2 N NaOH solution. Desiccated thyroid (250 mg.) was added to serve as a carrier. The thyroid was digested for six hours, after which the tissue digest was made up to 50 cc. A 5 cc. aliquot was removed for I^{131} count with a Victoreen tube and a Tracerlab Autoscaler. Forty ml. of the digest were used for the separation of thyroxine, diiodotyrosine and monoiodotyrosine (Gross *et al.*, 1950; Rosenberg, 1951), and inorganic iodine, according to the technique of Perlman, Chaikoff and Morton

*** Radioactive iodine (I^{131}) was supplied by the Atomic Energy Commission, Oak Ridge, Tennessee.

(1941). The radioactivity of each fraction was measured. A correction was made for disintegration, and calibration curves were made for absorption of radioactivity by the reagents used in the extraction.

Studies on blood: Two-tenths cc. of serum from each animal was dried and counted by means of the Victorren Geiger-Müller tube. A calculation was made for total serum I^{131} content in the animal, assuming that the total serum would be 5 per cent of body weight.

Studies on urine: An aliquot of the urine collected 4, 6, 12, 24, 48 and 96 hours after injection was counted. A correction was made for disintegration.

RESULTS

1. *Food intake:* As shown in Table 2, food intake was sharply decreased in all the groups of animals treated with cortisone acetate. This was true

TABLE 2. AVERAGE DAILY FOOD INTAKE PER RAT IN GROUPS A AND B

Experimental groups		Food intake grams per 24 hrs.		
		Before treatment with cortisone	During treatment with cortisone	
			After 1 week	After 3 weeks
Group A	Normal	21.50 gm.	19.50 gm.	20.00 gm.
	Normal + Cortisone		13.25 gm.	14.00 gm.
Group B	Normal	22.40 gm.	20.50 gm.	19.75 gm.
	Normal + Cortisone		15.00 gm.	14.50 gm.
	Adrenalectomized + DCA	—	19.00 gm.	22.00 gm
	Adrenalectomized + Cortisone + DCA		13.50 gm.	10.00 gm.

in both intact and adrenalectomized rats. There appeared to be no change in food intake in relation to duration of cortisone treatment.

2. *Influence of cortisone therapy on body weight:* Figure 1 shows the effect of subcutaneous cortisone therapy on the weight of normal rats and adrenalectomized rats maintained with DCA. They lost about 50 grams compared with their pretreatment weight, whereas the animals not treated with cortisone gained about 50 grams during the same time. The loss was similar in the normal treated rats and in the adrenalectomized treated rats. The weight curve of the adrenalectomized animals maintained with DCA was quite similar to that of the controls.

3. *Influence of cortisone therapy on weight of thyroid, adrenal glands and kidneys:* Thyroid weight was decreased in the cortisone treated groups. However, the average for 100 grams of body weight showed no significant difference (see Table 1). The weight of the adrenal glands was greatly de-

creased in the cortisone treated animals. The average weight of the kidneys per 100 grams of body weight seemed to be a little greater in the cortisone treated groups than in the untreated groups, although the change was not significant.

4. *Radioiodine uptake of the thyroid gland:* The I^{131} uptakes of the thyroids expressed as per cent of the injected dose are charted on Figure 2. The peak of uptake was reached 12 hours after injection.

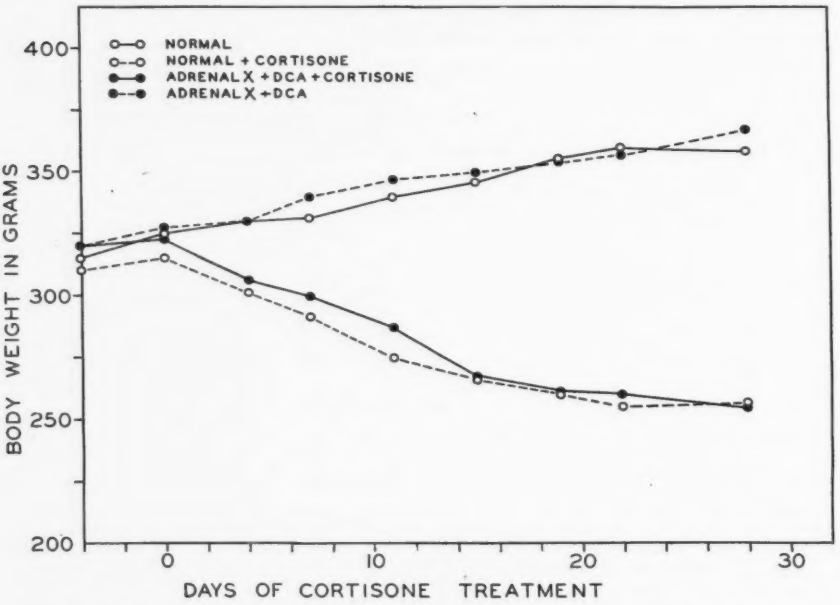

FIG. 1. Body weight curves of rats of group B. The normal rats and the adrenalectomized rats+DCA had very similar growth curves. The normal rats+cortisone and the adrenalectomized rats+DCA+cortisone had very comparable losses in body weight.

In the normal animals of Groups A and B, the maximum uptake was slightly more than 5% of the injected dose. These groups had body weights of 330 ± 18.1* grams and 358 ± 14.0* grams respectively. The normal rats of Group C had a mean body weight 262.5 ± 17.5 grams and showed a maximum uptake of 3.68% of injected I^{131}.

The normal animals treated with cortisone had an uptake fairly similar to that of the normal untreated rats of the same groups except that they showed slightly less uptake 24 hours after injection. The number of animals

* Mean body weight ± standard deviation.

at each period of time was too small to make a statistical study and to interpret the small variations obtained.

The adrenalectomized animals had an uptake quite similar to that of the controls of Groups A and B, but the adrenalectomized rats treated with cortisone had a definitely decreased uptake. The uptakes were not only

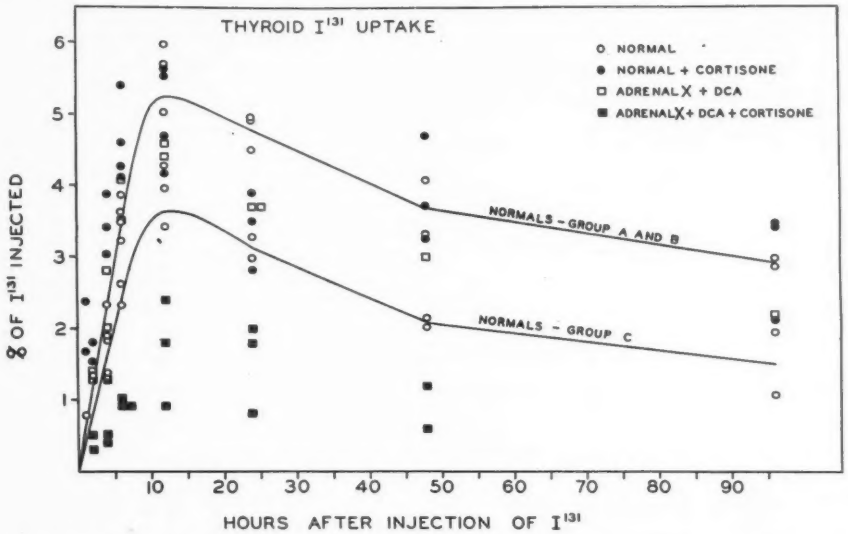

FIG. 2. Uptake of I^{131} by the thyroid in the different groups. Each point represents the uptake value of a single rat. The curves were constructed with average values of normal rats of Groups A and B (higher curve) and Group C (lower curve).

smaller than the counts of the controls of Groups A and B, but also smaller than the counts of the controls of Group C.

5. *Fractionation of the radioiodine of the thyroid gland:* In normal untreated rats, the radioactivity of the inorganic iodine fraction varied very little with time. It rose to 6–7% in the first 4 hours, and then increased very slightly to 9–10% 96 hours after injection. The radioactivity of the monoiodotyrosine and diiodotyrosine fraction reached 80% of the total I^{131} of the gland 1 hour after injection and stayed at that level for 2 to 4 hours. Then it decreased slowly to 50% after 96 hours. The radioactivity of the thyroxine fraction was 25% 12 hours after injection, 30% after 24 hours and 40% 96 hours after injection.

The different fractions were the same in the normal untreated rats, in the normal cortisone treated rats, in the adrenalectomized untreated rats and in the adrenalectomized cortisone treated rats (Figure 3).

6. *Radioiodine content of the serum:* Figure 4 shows the results obtained. The I^{131} concentration of the entire serum seemed to be the same in the normal rats, the normal cortisone treated rats and the adrenalectomized rats. This concentration decreased from 7% of the I^{131} injected (1 hour after

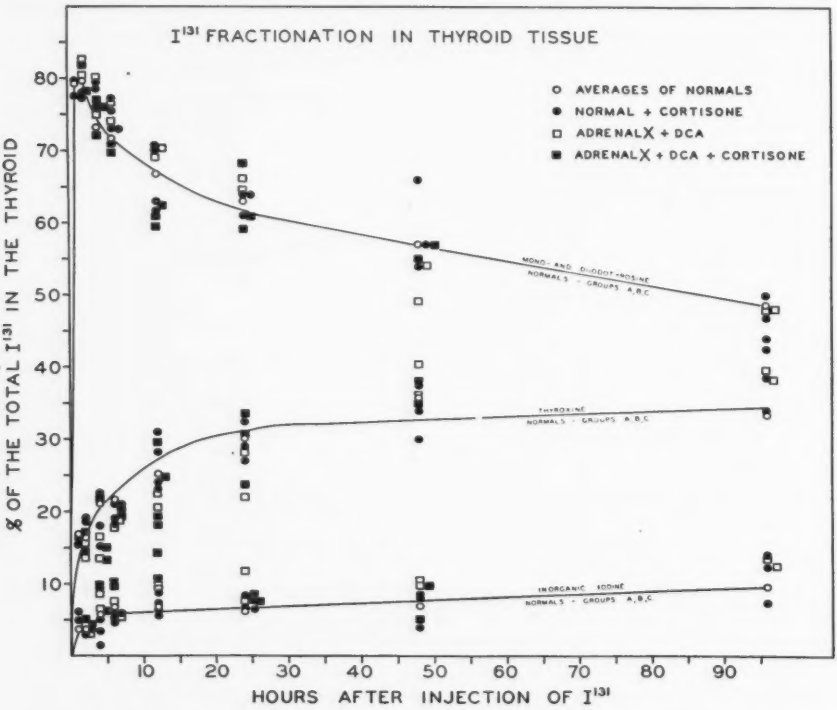

FIG. 3. I^{131} fractionation of thyroid tissue in the different groups. The curves were constructed using average values of each fraction as determined on thyroid tissue of normal rats of Groups A, B and C.

injection) to 0.25% (48 hours after injection). On the other hand, in the adrenalectomized rats treated with coritsone the I^{131} content of the serum was greatly increased just after I^{131} injection. This high I^{131} level persisted for 12 hours, after which it approached that found in the other groups of rats, although it was still higher than in the other groups.

7. *Excretion of I^{131}:* Figure 5 shows the amounts of I^{131} excreted expressed as per cent of the injected dose. It is clear that in the normal control rats not receiving an iodine free diet over 80% of the amount injected is excreted within 24 hours, and over 90% after 96 hours. This is also true for the normal rats treated with cortisone. It should be noted that the I^{131} excretion of the adrenalectomized untreated rats is slightly slower than

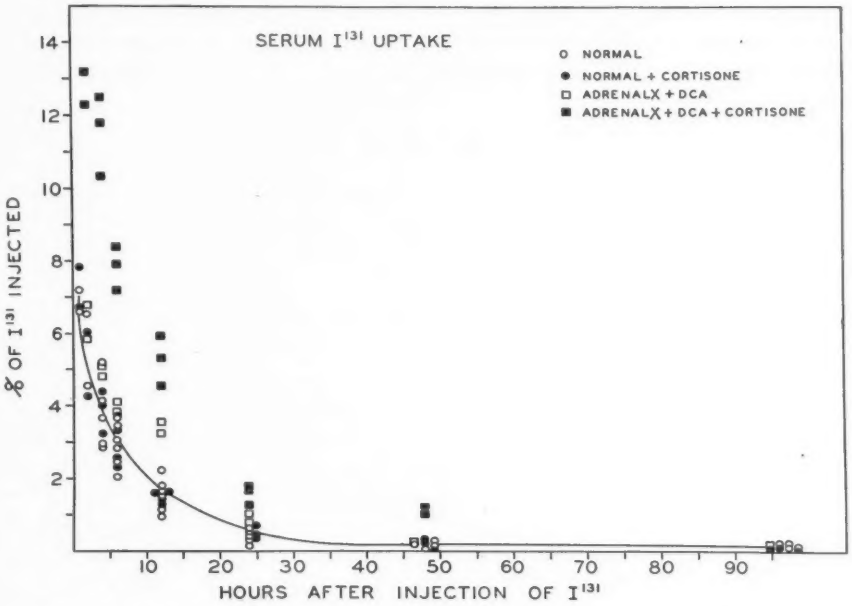

FIG. 4. I^{131} content of the entire serum. The curve was constructed using average values of normal rats of Groups A, B and C.

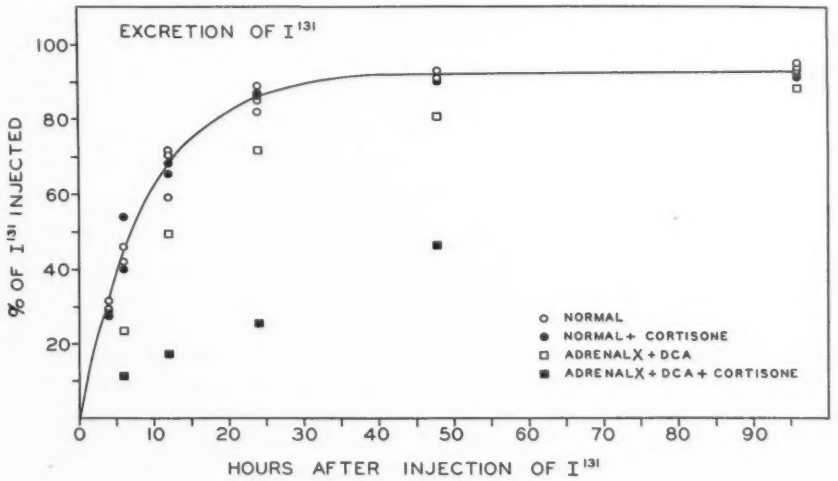

FIG. 5. Excretion of I^{131} in pooled urines and feces. The curve was constructed using average values of normal rats of Groups A, B and C.

that of the normal controls or the intact rats given cortisone. The adrenalectomized rats treated with cortisone had a much slower excretion: 46.5% after 48 hours. Unfortunately, the I^{131} excretion was not studied 96 hours after injection of I^{131}.

DISCUSSION

The experimental demonstration that cortisone alters thyroid function is attended with considerable difficulty. In the hands of other workers the study of I^{131} uptake has given variable results (Table 3) probably due

TABLE 3. COMPARISON OF EXPERIMENTAL CONDITIONS USED BY VARIOUS WORKERS IN STUDYING THE EFFECT OF CORTISONE THERAPY ON THE UPTAKE OF I^{131} BY THE THYROID OF INTACT RATS

Authors	Diet	Cortisone daily dosage	Duration of treatment	I^{131} injected (μ curies)	Time of study after I^{131} injection	I^{131} uptake of thyroid
Money et al. (1950)	Low Iodine	2.5 mg.	10 days	1	24 hrs.	% of injected dose: Controls 41.1% Cortisone 38.8%
		5.0 mg.	10 days	1	24 hrs.	% of injected dose: Controls 61.3% Cortisone 45.5%
		10.0 mg.	10 days	1	24 hrs.	% of injected dose: Controls 49.4% Cortisone 43.3%
Paschkis et al. (1950)	Normal	Adrenocortical extract 1 cc. every hour	for 7 hours	4	4 hrs.	Increased but not significantly.
Botkin et al. (1951)	Normal	2.5 mg.	One injection	5	2 hrs.	10.15% decreased below control values (probably not significant).
Money et. al. (1951)	High carbohydrates	2.5 mg.	10 days	1	24 hrs.	% of injected dose: Controls 31.2% Cortisone 11.1%
Perry (1951)	Low Iodine	5.0 mg.	6–7 days	15	24 hrs.	Slightly decreased. No change in I^{131} distribution in the thyroid.
Present Study	Normal	4.0 mg.	28 days	4	From 1 hr. to 96 hrs.	No significant change. No change in I^{131} distribution in the thyroid.

to differences in the iodine content of the diet and the amount and duration of cortisone therapy. Measurements of the ratio of thyroid to body weight (Winter *et al.*, 1950), or of the height of acinar epithelium (Higgins *et al.*, 1951) are also difficult to interpret in terms of thyroid function.

We were unable in intact rats to demonstrate that cortisone significantly decreased the uptake of I^{131}. The difference of this finding from that of Money (1950) may be due to the fact that we did not use an iodine-free diet. On the other hand, in adrenalectomized rats maintained on DCA, cortisone caused a definite decrease in I^{131} uptake. Possibly the thyroid is more sensitive to the effects of cortisone in the absence of the adrenal glands as suggested by Nathanson *et al.* (1940). The fact that in this group

of rats the diminished uptake of I^{131} was accompanied by diminished excretion in the urine and feces and increased concentration in the blood remains unexplained, since we were unable to demonstrate histological changes in the kidneys. However, the possibility that there was an impairment of the renal function cannot be excluded. We were unable to demonstrate any alterations in the various iodine fractions of the thyroid glands in either the cortisone treated or adrenalectomized animals, thereby confirming the finding of Perry (1951).

SUMMARY

Cortisone acetate, 4 mg. per day subcutaneously, was given to male adult rats for a period of 28 days. The rats had been divided into two groups: (1) Intact, and (2) Adrenalectomized and maintained on DCA pellets. All the rats received a diet containing 1.2 mgs. of iodine per 100 grams of food. At the end of the treatment, thyroid function was studied by means of I^{131} uptake. The essential findings were:

1. The I^{131} uptake in normal rats treated with cortisone and in adrenalectomized rats maintained with DCA was not significantly changed compared with controls. Adrenalectomized rats maintained with DCA and treated with cortisone showed diminished I^{131} uptake.

2. In none of the groups was there found any difference in the proportions of the thyroxine, mono- and diiodotyrosine, and inorganic iodine fractions of I^{131} in thyroid tissue.

3. The adrenalectomized rats, maintained with DCA and treated with cortisone, showed in addition to a decreased uptake of I^{131} by the thyroid gland:

   (a) Diminished excretion of I^{131} in the urine and feces during the 48 hours following the I^{131} injection (the other groups showed no difference from normal).

   (b) Increased I^{131} concentration in blood, at least during the first 12 hours following the I^{131} injection.

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HISTO-PHYSIOLOGICAL EFFECTS OF CORTISONE ON RAT PITUITARY AND THYROID[1]

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THE present investigation was undertaken to ascertain the effects of cortisone treatment on the cellular composition of the rat adenohypophysis. In view of recent evidence for the influence of adrenal cortical steroids on the thyroid, the latter organ was also examined morphologically and its functional state was assessed by determining plasma and thyroid protein-bound iodine (PBI).

MATERIALS AND METHODS

One shipment of young adult male Sprague-Dawley rats was used in this study. In one experiment 12 rats received daily injections of 5 mg. cortisone acetate[2] for 20 days. They were given ground Purina Laboratory Chow and tap water ad libitum. Since recent observations (Halmi, 1952 b) indicated that a rigid quantitative and qualitative control of the food intake of rats used in pituitary and thyroid studies is made necessary by the potential goitrogenicity of commercial regimens, each experimental animal was matched with an uninjected control which was pair-fed the same batch of diet. Twenty-four hours after the last injection the rats were anesthetized with ether, and as much blood as possible withdrawn from the abdominal aorta, using oxalate as anticoagulant. The plasma PBI values were determined using the method of Barker (1948). The pituitaries were fixed without previous weighing in a fluid containing 9 parts SUSA and 1 part saturated aqueous picric acid solution. The thyroid lobes were dissected out separately and weighed on a torsion balance. One lobe was fixed together with the pituitary, the other was homogenized in dilute sulfuric acid. An aliquot of the thoroughly suspended homogenate was taken for determination of thyroid gland PBI. A suitable amount of low-iodine protein, usually dog plasma, was added as an organic "carrier" to prevent loss of iodine. The other endocrine organs, the spleen and the left kidney were also weighed.

In a second experiment 15 rats were started on daily injections of 2 mg. cortisone with 7 uninjected rats serving as controls. Seven injected rats died before the experiment was terminated. The autopsy findings were cachexia, purulent pneumonia, empyema and metastatic abscesses in the liver, the kidneys and the diaphragmatic peritoneum. Two control rats died owing to accidental causes. The injected rats received Purina Laboratory Chow and tap water ad libitum, whereas the daily intake of the same diet by the control group was so restricted as to equal the amount ingested by an identical number

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[2] Cortisone (Cortone acetate Merck) was generously supplied by Dr. A. Gibson, Merck & Co., Rahway, N.J.

of rats in the injected group. The experiment was carried on for 83 days and the animals were sacrificed by etherization on the following day. Only morphological studies were performed on these rats. Their organs were weighed and their pituitaries and thyroids fixed as described above. All pituitaries were sectioned in the horizontal plane at 4 μ and stained with the technique of Halmi (1952 c). To occasional glands the periodic acid-leucofuchsin method (Purves and Griesbach, 1951 a) was also applied. Two thousand cells were counted in two sections from each pituitary. The thyroids were cut at 6 μ and central sections were stained with Gomori's trichrome stain.

RESULTS

1. *Organ Weights*

The initial and final body weights and the organ weights of the rats treated with 5 mg. of cortisone daily for 20 days and those of their pair-fed controls are presented in table 1. The most outstanding differences be-

TABLE 1. BODY AND ORGAN WEIGHTS IN CORTISONE TREATED AND CONTROL RATS

Group	Initial body wt.	Final gm.	Testes	Left kidney	Seminal vesicles	Ventral prostate	Thymus	Spleen	Adrenal	Thyroid
			Wt. mg.							
5 mg. cortisone per day for 20 days (12)*	230±2	192±4§	3135†	950	272	256	47	301	15.6	14.6
			1635	495	142	135	24.5	160	8.2	7.7
			±40‡§	±11§	±6	±5	±3§	±9§	±0.4§	±0.4
Pair-fed controls (12)	231±3	247±5	3192	929	334	302	357	565	41.9	17.1
			1291	377	136	124	144	228	16.9	7.0
			±41	±8	±5	±6	±8	±7	±0.4	±0.4
2 mg. cortisone per day for 83 days (8)	209±3	220±8*	3127	999	426	391	80	385	24.1	22.8
			1445	455	193	175	31.4	170	10.8	10.4
			±77	±12§	±6§	±17	±3§	±5§	±1.0§	±1.7
Controls (5)	211±5	253±3	3142	1012	356	381	137	802	45.5	21.3
			1245	401	140	149	54.2	316	18.2	8.4
			±71	±6	±11	±16	±8	±25	±0.4	±0.5

* Number of rats per group.
† Absolute weight.
‡ Weight per 100 gm. body weight: mean ± standard error.
§ Difference from control value, statistically significant at 1% level of confidence.

tween the two groups were in their final body weight, adrenal, thymic and splenic weight, the values for the treated animals being significantly lower. A reliably higher organ-body weight ratio was found for the kidneys and the testes of the cortisone-injected animals. The reduction in the weights of the seminal vesicles, ventral prostate and thyroid of the treated animals was proportionate to the loss of body weight. The thyroid weights were within normal limits.

The body and organ weight data for the rats treated with 2 mg. of cortisone daily for 83 days and their controls are also summarized in table 1. They are essentially in agreement with those of the previous group but for the fact that a) there was no weight loss but only a significantly diminished weight gain in the treated animals of this experiment, b) their adrenal atrophy was less profound and c) their thyroid-body weight and seminal vesicle-body weight ratios were somewhat above the normal range. The

conspicuous reduction of the thymic weight in this control group can be interpreted as being due to the combined effect of age and prolonged undernutrition.

2. *Pituitary Cell Counts*

The adenohypophysial cell counts of the 5 mg. and 2 mg. groups and their respective controls are summarized in table 2. There was no significant numerical difference between the acidophils of the treated and the control rats in either experiment. Cytologically the acidophils of the cortisone-injected rats appear to stain somewhat more brilliantly with orange G. The beta cell counts were likewise of the same order of magnitude in all four groups. In the cortisone-treated rats, however, there appeared to be a higher incidence of enlarged, spherical beta cells with decresed affinity of

TABLE 2. PITUITARY CELL COUNTS IN CORTISONE TREATED AND CONTROL RATS

Group	Acidophils	Chromophobes	Beta cells§	Delta cells§	Vacuolated§	
					Beta cells	Delta cells
5 mg. cortisone per day for 20 days (12)*	44.60±0.70†	44.55±0.42	3.15±0.41	8.15±0.31‡	0.03±0.03	0.40±0.15
Pair-fed controls (12)	45.30±0.94	45.27±0.57	3.55±0.31	6.90±0.48	0.08±0.04	0.17±0.06
2 mg. cortisone per day for 83 days (8)	43.89±0.76	46.25±0.68	3.54±0.49	6.16±0.45	0.36±0.11	0.54±0.15
Controls (5)	42.91±1.37	48.08±0.98	3.09±0.38	5.94±0.38	0.01±0.00	0.12±0.03

* Number of rats per group.
† Mean ± standard error.
‡ Difference from control value statistically significant at 5% level of confidence.
§ The terminology applied to pituitary basophils has been discussed in a previous note (Halmi, 1952 a).

their granules for aldehyde-fuchsin, and the 2 mg. cortisone group displayed an abnormally high count of vacuolated beta cells. Whereas the delta counts of the 2 mg. rats and their controls were not significantly different and in the normal range, the number of delta cells in the group treated with 5 mg. of cortisone was somewhat higher than that of the controls and definitely above the physiological range of variation. The incidence of vacuolated delta cells was distinctly elevated in both cortisone treated groups. The cytological appearance of the majority of these cells was that of thyroidectomy cells and their negative reaction with the periodic acid-leucofuchsin stain (Purves and Griesbach, 1951a) supported this interpretation.

3. *Thyroid Histology*

The histological picture of the thyroids of both cortisone-treated groups and their respective controls is shown in figures 1–4. Whereas the glands of all untreated animals displayed medium sized or large follicles lined by cuboidal or only slightly higher epithelium and containing dense colloid

with frequently highly acidophilic staining properties, the thyroid glands of the experimental animals had small follicles lined by columnar or tall columnar epithelium enclosing little or no colloid.

4. *Protein-bound Iodine Determinations*

A PBI value of 5.69 ± 0.59 μg./10 mg. tissue was found in the thyroids of the rats injected with 5 mg. of cortisone daily for 20 days. The corresponding value for their controls was 7.80 ± 0.61 μg. The difference is significant at the 5% level of confidence. The plasma PBI in the rats given 5 mg. of cortisone daily was 3.31 ± 0.26 μg. % vs. 2.67 ± 0.16 μg. % of the controls.

DISCUSSION

Although the adrenal of the cortisone treated animals of this study presented the familiar picture of compensatory atrophy, and there is good reason to believe that the latter is brought about by a decreased discharge of ACTH from the pituitary (Sayers, 1950, Stebbins, 1951), there was no change in their pituitaries which could have been correlated with the profound alteration of the adrenocorticotrophic function. This finding is in agreement with the observations of Baker (1951), who failed to encounter any significant change in the hypophyses of rats whose endogenous corticoid production had been tremendously augmented by the administration of massive doses of ACTH. Winter *et al.* (1950) also commented on the lack of changes similar to those characteristic of human hyperadrenocorticism (Crooke's hyalinization of pituitary basophils) in the hypophyses of rats treated with 3 mg. of cortisone for 42 days. These negative observations tally with the results which indicated that ACTH in the rat pituitary is not stored in the form of microscopically visible granules (Halmi and Bogdanove, 1951 a,b).

On the other hand, the pituitaries of the cortisone injected animals showed some evidence of an increased rate of thyrotrophin (TSH) release. The histophysiology of TSH secretion in the rat has been considerably clar-

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PLATE 1. Photomicrographs of representative central fields of thyroids stained with Gomori's trichrome method. Green filter, magnification 125×.

FIG. 1. Thyroid of pair-fed control of 5 mg. cortisone-group. Cuboidal epithelium, many follicles distended by dense, acidophilic colloid.

FIG. 2. Thyroid of rat treated with 5 mg. of cortisone daily for 20 days. Columnar epithelium, follicles contain only very little dilute colloid.

FIG. 3. Thyroid of control of 2 mg. cortisone-group. Histological picture similar to that seen in fig. 1.

FIG. 4. Thyroid of rat treated with 2 mg. of cortisone daily for 83 days. Histological picture similar to that seen in fig. 2.

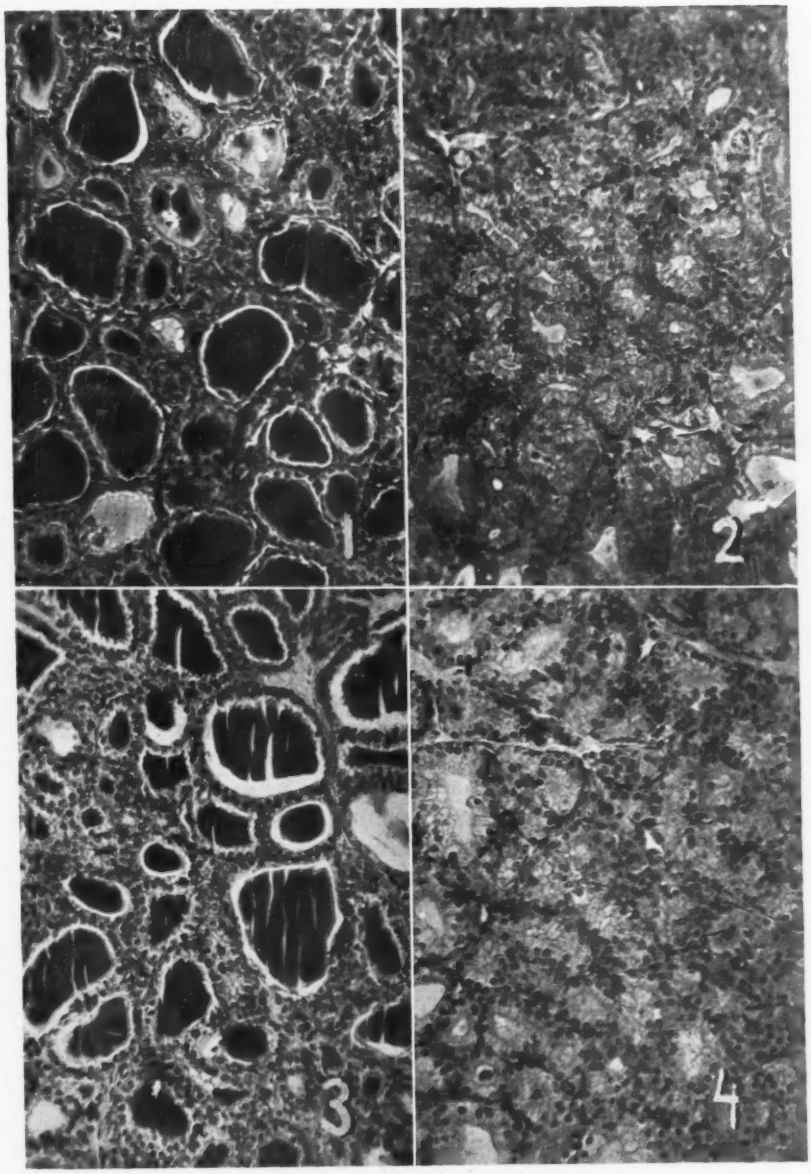

PLATE I

ified by recent investigations. There is good evidence that the aldehyde-fuchsin (AF) staining granules of the beta cells contain TSH (Purves and Griesbach, 1951 b, c, Halmi, 1952 b). If TSH is released at an increased rate the beta cells become hyperplastic, enlarged and partly vacuolated, thereby gradually losing the affinity of their granules for aldehyde-fuchsin (Halmi 1952 a). Eventually they are transformed into the vacuolated thyroidectomy cells which are AF negative, and thus the number of non-AF staining (delta) basophils is increased. In contrast to the equally AF negative vacuolated castration cells the granules of the thyroidectomy cells do not stain with periodic acid-leucofuchsin (Purves and Griesbach, 1951 a). In the cortisone treated rats of this study the following histological stigmata of increased TSH release were present: a) enlargement and pale AF staining of some of the beta cells, b) the presence of an unusually high number of vacuolated beta cells in the 2 mg. group, c) the occurrence of an increased number of AF and periodic acid-leucofuchsin negative thyroidectomy cells (vacuolated delta cells of table 2) in both groups and d) the elevated delta count in the 5 mg. group.

The thyroids of our cortisone injected rats, as compared with those of the controls, also presented obvious histological signs of a higher level of thyrotrophic stimulation. The low thyroid PBI of the group injected with 5 mg. of cortisone daily was in keeping with the reduced amount of colloid found in these glands. Earlier investigators had reported no change (Winter *et al.*, 1950) or an inactivation (Mercier-Parot and Tuchmann-Duplessis, 1951) of thyroid structure following the administration of cortisone. Using thyroid I^{131} uptake as a functional test, a number of workers have encountered a definite depressing effect of cortisone on thyroid function in the rat (Money *et al.*, 1950, Money *et al.*, 1951, Verzár and Vidovič, 1951, Perry, 1951) and in man (Hill *et al.*, 1950, Wolfson *et al.*, 1950, Frederickson, 1951). Woodbury *et al.* (1951) also established that the effect of exogenous TSH in hypophysectomized rats, as judged by the thyroid uptake of I^{131}, was partly counteracted by cortisone, while the action of the latter on PBI^{131} was equivocal. The interpretation of "corticogenic hypothyroidism" as the result of a block of pituitary thyrotrophic activity (Wolfson *et al.*, 1950, Hill *et al.*, 1950) was challenged by Perry (1951), who found that the rate of iodine discharge from the thyroid, which is also under the control of TSH, was not altered in the cortisone treated rats.

The present study appears to rule out the following points of attack of cortisone in exerting its thyroid depressing activity: a) on the formation of TSH, since the pituitaries of the cortisone treated animals contained approximately normal amounts of beta granular material, which is believed to be the morphological sign of TSH storage, b) on the discharge of TSH,

since the pituitaries showed cytological changes which were indicative of an increased rate of release of TSH, and c) on the action of TSH on the thyroid, since the thyroids of the cortisone treated animals, far from exhibiting an inactive histological picture, actually showed evidence of a higher level of thyrotrophic stimulation than that of the control glands. Recent observations on hypophysectomized rats treated with TSH alone or in conjunction with cortisone (Halmi, 1952 d) also showed that, contrary to the findings of Woodbury *et al.* (1951), on I^{131} uptake, the morphological effects of TSH on the thyroid gland were not hampered by simultaneously administered cortisone.

The pathogenesis of this thyroid hyperplasia in cortisone treated rats is difficult to explain. The role of dietary goitrogens, which was blamed for the occurrence of similar thyroid changes in a group of untreated rats (Halmi 1952 b), can be excluded because of the failure of the pair-fed controls to show these alterations. The assumption of a cortisone induced reduction in the rate of thyroid hormone formation with a subsequent compensatory hypersecretion of TSH would be consistent with the previous observations of a decreased I^{131} uptake by the thyroid in consequence of cortisone administration. The possible mechanisms of such an interference include a) alterations in the extrathyroidal metabolism of iodide, b) a depression of iodide uptake by the thyroid, and c) a true goitrogenic (thiouracil-like) effect of the steroid. However, the plasma PBI in our rats on 5 mg. of cortisone daily was found to be somewhat, although not significantly, higher than that of the controls. In this connection it is of interest that according to a recent study of Goldberg and Chaikoff (1951) plasma PBI does not appear to be the decisive factor in the regulation of TSH formation and output. It may also be that, whatever handicap cortisone imposed on the thyroid, this had been overcome in our rats through thyrotrophic overstimulation by the time the plasma PBI was examined.

SUMMARY

1. Young adult male albino rats were treated with 5 mg. of cortisone daily for 20 days and with 2 mg. daily for 83 days, respectively. Their controls were pair-fed.

2. No change was encountered in their pituitaries which could have been correlated with the substantially decreased output of ACTH reflected by the atrophy of the adrenals. On the other hand, there was some cytological evidence in the hypophyses of both cortisone treated groups for an increased rate of TSH release.

3. The histological structure of the thyroids of the treated animals was indicative of a higher level of thyrotrophic activation than that occurring

in the controls. The PBI of the thyroids in the 5 mg. group was lower than that of the control glands. The plasma PBI was somewhat higher in the rats treated with 5 mg. of cortisone daily than in their controls.

4. It is concluded that the cortisone-induced inhibition of thyroid function, which was encountered by several previous workers, cannot be due to an interference with the production or action of TSH. On the contrary, the data presented in this paper suggest a promoting effect of cortisone on the thyrotrophic function of the pituitary.

5. The possible mechanisms of this action are discussed.

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NUTRITIONAL FRACTIONS AFFECTING SURVIVAL IN YOUNG ADRENALECTOMIZED RATS*

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INTRODUCTION

IN PREVIOUS studies (Ralli and Graef, 1943; Ralli, 1946; Dumm and Ralli, 1948) on young adrenalectomized rats, it was observed that survival following adrenalectomy was significantly influenced by the level of calcium pantothenate in the diet. An intake of at least 4 mg. daily of calcium pantothenate was necessary to obtain prolonged survival. With this intake, 50% of the adrenalectomized rats survived for more than 75 days. When sodium chloride was completely omitted from the diet survival was sharply curtailed, regardless of the amount of calcium pantothenate ingested. The studies were done on 60 day old rats that had been for 30 days on a diet deficient in pantothenic acid. The present report concerns the effects of other fractions of the vitamin B complex and of ascorbic acid on the survival of rats similarly treated. The survival of rats never subjected to a period of pantothenate deficiency was also determined. In view of the importance of sodium chloride to the adrenalectomized rat, the amounts of sodium chloride necessary for prolonged survival on the high pantothenate intake were measured.

EXPERIMENTAL

Male and female rats were placed on the pantothenate deficient diet when they were between 30 and 35 days of age, at which time they weighed approximately 65 gm. The composition of the diet was as follows: vitamin-free casein 22%, sucrose 62%, hydrogenated cotton seed oil 5%, salt mixture exclusive of sodium chloride 5% and cod liver oil 2%. To this basic diet the following supplements were added per 100 gm. of diet: thiamine 0.3 mg., pyridoxine 0.3 mg., riboflavin 0.5 mg., folic acid 0.1 mg., biotin 0.05 mg., niacin 1.0 mg., choline chloride 30 mg., inositol 5 mg., mixed tocopherols 7.5 mg., synthetic vitamin K 5 mg. (2-methylnaphthohydroquinone-1,4-tetra sodium diphosphate). The rats were continued on the diet for 30 days and were then adrenalectomized. Following adrenalectomy, except in the group of rats continued on the pantothenate deficient diet, the diet was supplemented with 0.1 mg. of calcium pantothenate per 10 gm. of diet. The rats consumed approximately 10 gm. of food daily. This amount of

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calcium pantothenate is adequate for normal rats of this age. Altogether, 11 groups of rats were studied. The rats in Group 2 did not receive an excess of any fraction but did receive, following adrenalectomy, 0.1 mg. of calcium pantothenate per 10 gm. of diet. In the rats in Groups 3 to 9, inclusive, the diet was supplemented with an excessive amount of the fraction of the vitamin B complex tested, as indicated in Table 1. The rats in Group 10 received an excessive amount of ascorbic acid following adrenalectomy. The rats in Group II received an amount of calcium lactate equivalent in its calcium content to the calcium contained in the pantothenate given to the rats in Group 8.

In order to evaluate the effects of different amounts of pantothenate on the survival of adrenalectomized rats *never* subjected to pantothenate deficiency, two groups of 50

TABLE 1. INFLUENCE OF EXCESSIVE AMOUNTS OF VARIOUS NUTRITIONAL SUPPLEMENTS ON THE SURVIVAL OF ADRENALECTOMIZED RATS‡

All rats adrenalectomized after a 30 day period of pantothenate deficiency

Group number	Pantothenate mg./10 g. diet	Excessive supplement		Number of rats	Per cent surviving			Median survival	Survival index*
		Substance	mg./10 g diet		10 days	25 days	50 days	days	
1	none	none	—	13	15	0	0	7	1.0
2	0.1	none	—	27	52	44	30	11	1.4
3	0.1	Thiamine	2.0	21	43	19	14	10	1.4
4	0.1	Riboflavin	1.5	13	62	38	31	11	1.8
5	0.1	Folic Acid	0.3	30	70	47	37	17	2.1
6	0.1	$B_{12b}+B_{12}$ Conc.†	0.005	54	52	48	43	24	2.4
7	0.1	Pyridoxine	2.0	14	29	14	14	6	0.5
8	4.0	Calcium Pantothenate	4.0	25	80	68	56	>50	>5.0
9	0.1	Biotin	0.15	19	74	68	63	>50	>5.0
10	0.1	Ascorbic Acid	3.0	29	62	52	52	>50	>5.0
11	0.1	Calcium Lactate	2.8	16	56	38	31	17	1.6

* The survival index is the ratio of the median survival time observed in each experimental group to the expected median survival of adrenalectomized rats of the same average weight (± 10 grams) continued after adrenalectomy on a diet deficient in calcium pantothenate.
† B_{12} Concentrate supplied by the Lederle Laboratories.
‡ All rats were allowed free access to a solution of 1% sodium chloride.

day old rats were studied. These rats were kept on the normal laboratory diet prior to adrenalectomy. The dog chow used for the laboratory diet contains approximately 0.1 mg. of pantothenate per 15 gm. of diet. Following adrenalectomy, one group of animals was kept on the diet containing 0.1 mg. of pantothenate per 10 gm. of diet and the other animals were given the diet containing 4 mg. of pantothenate per 10 gm. of diet.

The effects of giving varying amounts of sodium chloride to adrenalectomized rats on the high pantothenate diet were studied in 62 rats. Sodium chloride was given as a 0.167 M solution in amounts which provided 1, 3, 5 or 6 m-mols of sodium chloride per rat daily. A 6th group of rats was allowed free access to a 1% solution of sodium chloride as drinking water. In the rats on the measured amounts of sodium chloride, a separate drinking bottle of distilled water was placed in the cage so that no limitation of fluid intake was imposed.

In all experiments the survival of the rats was followed for at least 50 days. At this time the completeness of adrenalectomy in the surviving animals was confirmed by withdrawing salt from the drinking water.

RESULTS

The effect of the excessive amounts of the various fractions of the vitamin B complex, of ascorbic acid and of calcium lactate on the survival of

these young adrenalectomized rats is given in Table 1. Survival is reported as the per cent of the group alive at 10, 25 and 50 days after adrenalectomy, and as the median survival of the group. The survival index was calculated for the various groups. This index is the ratio of the median survival time observed in each group to the expected median survival of adrenalectomized rats of the same average weight (± 10 gm.) continued on a diet deficient in pantothenate after adrenalectomy (Dumm and Ralli, 1948). The data indicate that excessive amounts of calcium pantothenate, biotin and ascorbic acid will significantly prolong the survival of young adrenalectomized rats subjected to a preliminary period of pantothenate deficiency. Both folic acid and the vitamin B_{12} concentrate prolonged survival

TABLE 2. SURVIVAL OF ADRENALECTOMIZED RATS NEVER SUBJECTED TO PANTOTHENATE DEFICIENCY

Pantothenate content of diet mg./10 g. diet	Number of rats	Per cent surviving		
		10 days	25 days	50 days
0.1	50	64	54	48
4.0	28	89	75	75

following adrenalectomy, but the effects were not as consistent or as pronounced as with the three fractions just mentioned.

The calcium content of the calcium pantothenate was not a factor in prolonging survival. Calcium lactate, in amounts equivalent to the amounts in the pantothenate, had no significant influence on survival following adrenalectomy. Excessive amounts of pyridoxine added to the diet after adrenalectomy curtailed survival. Further study of this situation showed that decreased survival on a high pyridoxine intake occurred only when rats were adrenalectomized after a previous period of pantothenate deficiency. Seven of a group of 8 adrenalectomized rats, never deficient in pantothenate, survived more than 50 days on an excessive intake of pyridoxine, while 9 of a group of 10 intact rats transferred to the high pyridoxine diet after a 30 day pantothenate deficiency were alive after 50 days on the high pyridoxine diet.

In Table 2 are given data on the survival of adrenalectomized rats never subjected to a period of pantothenate deficiency. Fifty days after adrenalectomy, 75% of the rats on the high pantothenate intake were alive, compared to 48% of the rats on the lower but adequate intake of pantothenate. Obviously, the survival of adrenalectomized rats on a normal diet can be improved by the addition of excessive amounts of pantothenic acid to the diet.

Table 3 shows the effect on survival of varying the sodium chloride intake of adrenalectomized rats on the high pantothenate diet. The data

show that at least 6 m-mols of sodium chloride are necessary daily for prolonged survival on the high pantothenate diet. When rats are allowed a 1% sodium chloride solution in their drinking water ad lib, the sodium chloride intake varies considerably; some rats will take in as much as 8 m-mols of sodium chloride daily, and almost all the rats will exceed 5 m-mols of sodium chloride daily. At 3 m-mols of sodium chloride, or less, per day, survival is sharply curtailed following adrenalectomy, even on the high pantothenate intake. The animals on the 1 m-mol intake diluted the sodium chloride to about half the original concentration by the distilled water which they also ingested. At 3 m-mols, or above, the rats ingested very small amounts of distilled water. When the sodium chloride intake was

TABLE 3. EFFECT OF NACL INTAKE ON THE SURVIVAL OF RATS ADRENALECTOMIZED AFTER A 30 DAY PANTOTHENATE DEFICIENCY AND THEN PLACED ON THE HIGH PANTOTHENATE DIET

NaCl intake m-mols/rat/day	Number of rats	Per cent surviving			Median survival days
		10 days	25 days	50 days	
1.0	10	30	0	0	8
3.0	8	75	0	0	14
5.0	8	100	50	25	26
6.0	11	82	73	36	39
ad libitum	25	80	68	56	>50

increased to 5 m-mols, 25% of the rats survived for 50 days; at 6 m-mols survival at this time had increased to 36%. When the rats were allowed free access to the sodium chloride solution, 56% survived for 50 days. These data confirm the importance of sodium chloride to the adrenalectomized rat and indicates that the level of pantothenate in the diet does not influence the requirement for a high salt intake.

DISCUSSION

The fact that the survival of completely adrenalectomized young rats can be influenced by nutritional factors suggests that the accompanying modification of the intracellular situation may partially compensate for the hormonal loss following adrenalectomy. In view of the fact that large amounts of sodium chloride were also necessary for prolonged survival it seems probable that the nutritional factors exert their effects on some aspect of carbohydrate or protein metabolism, rather than on the rat's capacity to handle salt and water. Other evidences of interaction between vitamins and hormones have been reported by several investigators (Morgan, 1951; Meites, 1952).

Although the pre-adrenalectomy diet in our experiments was deficient only in pantothenic acid, prolonged survival following adrenalectomy was

obtained with excessive amounts of biotin or ascorbic acid, as well as with pantothenate. Excessive amounts of pyridoxine were actually toxic for adrenalectomized rats under the conditions of the experiments. Emerson and Wurtz (1944) have reported that in rats there is an interdependence in the requirements for pantothenic acid and biotin. Deficiencies in either pantothenate or biotin will impair the utilization of pyruvate in the tissues of rats and ducklings (Olson and Kaplan, 1948; Olson *et al.*, 1948). Pantothenic acid, as part of coenzyme A, has been reported to have a catalytic function in citric acid synthesis (Stern and Ochoa, 1949; Novelli and Lipmann, 1950). Biotin has been implicated in the synthesis of the dicarboxylic acids by CO_2 fixation (Ochoa *et al.*, 1947; Blanchard *et al.*, 1950). Pyridoxal phosphate has been reported to function as a coenzyme for the transaminase enzyme system (Schlenk and Snell, 1945; Lichstein *et al.*, 1945; Green *et al.*, 1945).

It is suggestive that these three vitamins, each of which was found to influence strikingly the survival of adrenalectomized rats, all participate in reactions involving metabolities of the tricarboxylic acid cycle, a series of reactions which is believed to be a primary pathway of oxidative metabolism. It may be that the tricarboxylic acid cycle is one site of the biochemical disturbance in the adrenalectomized animal, and that vitamins which exert particularly striking effects on adrenalectomized animals do so because of their role in this cycle of reactions.

Umbreit (1951) has recently discussed the role of the adrenal hormones in enzymatic reactions in rat tissues. The oxidation of several substrates of the citric acid cycle was studied in kidney homogenates prepared from normal, adrenalectomized and adrenalectomized-cortisone treated rats. After adrenalectomy an increased requirement for adenylic acid and a decreased capacity to oxidize proline were demonstrated. Cortisone treatment tended to restore these values to normal. Proline oxidation in the liver was not influenced by adrenalectomy or cortisone. Much further work will be necessary to establish the possible effects of the adrenal hormones on tissue enzymes.

Decreased excretion of ascorbic acid by the rat has been reported previously during pantothenic acid deficiency and by adrenalectomized and hypophysectomized rats (Dumm and Ralli, 1949; Dumm, Ralli and Graef, 1952). While the rat synthesizes ascorbic acid, the possibility exists that a relative deficiency may have been produced in the rats in this study due to the combined effects of pantothenate deficiency and adrenalectomy. An excessive intake of ascorbic acid by the adrenalectomized rat would tend to restore or maintain the conditions necessary for the function of the intracellular enzymes which are dependent on adequate concentrations of ascorbic acid in the tissues.

The extent to which a nutritional factor can compensate for the loss of the adrenal hormones depends, in part, on the nature and the degree of the stress to which the animal is subjected. In a later paper we will report the effects of various types of stress on these long surviving adrenalectomized rats treated with excessive amounts of certain nutritional fractions.

SUMMARY

Thirty day old rats were given a diet deficient in pantothenic acid for 30 days, and were then adrenalectomized. After adrenalectomy the diet was supplemented with adequate amounts of pantothenate and with excessive amounts of one fraction of the vitamin B complex or of ascorbic acid. When the diet was supplemented with excessive amounts of calcium pantothenate, biotin or ascorbic acid, more than 50% of the rats survived for 50 days. On the control diet, which contained adequate but not excessive amounts of all fractions of the vitamin B complex, 30% of the rats survived for 50 days, with a median survival of 11 days. The median survival of rats on excessive amounts of pyridoxine following adrenalectomy was only 6 days. Excessive pyridoxine was not toxic to intact pantothenate deficient animals or to adrenalectomized rats not previously deficient in pantothenic acid. When calcium lactate was added to the diet in amounts containing calcium equivalent to that contained in the excessive dose of calcium pantothenate, the median survival was 17 days. Excessive amounts of a vitamin B_{12} concentrate improved survival, but less consistently than did pantothenate, biotin or ascorbic acid.

On the high pantothenate diet at least 6 m-mols of sodium chloride per rat were required daily.

Excessive calcium pantothenate also improved the survival of rats adrenalectomized at 60 days of age which had never been subjected to pantothenate deficiency.

It is suggested that changes in the intracellular situation, brought about by the administration of excessive amounts of pantothenic acid or biotin or ascorbic acid, contributed towards the prolonged survival of these completely adrenalectomized young rats.

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THE EFFECT OF VARIOUS HORMONES UPON THE HEPATIC SYNTHESIS OF CHOLESTEROL IN RATS*

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DESPITE the profound influence of thyroid activity upon cholesterol metabolism (Rosenman, Friedman and Byers, 1951, 1952; Friedman, Byers and Rosenman, 1952; Byers, Friedman, and Rosenman, in press), there is little evidence that similar influence is exerted by other endocrine glands. More recently, however, changes in serum cholesterol have been described following administration of adrenocorticotrophic and adrenocortical hormones (Conn, Vogel, Louis and Fajans, 1950; Adlersberg, Schaeffer and Drachman, 1950). It therefore was believed important to determine whether these and other hormones altered hepatic synthesis of cholesterol.

Previous studies from this laboratory clearly have shown that the liver is the main source of the plasma cholesterol content (Friedman, Byers and Michaelis, 1951), and that the biliary concentration of cholesterol is a reliable index of the rate of hepatic synthesis of this substance (Byers and Friedman, 1952). Therefore, it is believed that studies of the effects of various substances upon *in vivo* hepatic synthesis of cholesterol readily can be accomplished by determining their effect upon biliary cholesterol concentration, the technic employed in the present investigations. Observations also were made of the effect of various hormonal preparations upon biliary cholic acid content, since recent evidence has demonstrated an important relationship between endogenous cholate and cholesterol metabolism (Friedman and Byers, 1951, 1952; Byers and Friedman, 1952; Friedman, Byers and Rosenman, 1952).

METHODS

Ten weeks old male rats (Long-Evans strain) were fed a stock ration during a three week interval in which various hormonal preparations were administered subcutaneously.

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Ten rats received estradiol benzoate in sesame oil (Progynon-B),* 10 received testosterone propionate in sesame oil (Oreton),* and 10 received progesterone (Proluton).* Injections were given three times weekly. The dosage used per injection was: estradiol benzoate, 0.5 mg.; progesterone, 5 mg.; and testosterone propionate, 5 mg. Ten rats received adrenocortical extract in cottonseed oil (Lipoadrenal cortex),* 0.25 ml. three times weekly. Ten rats received daily injections of 1 mg. of adrenocorticotrophic hormone (equivalent to 2 mg. IA 1A standard) (ACTH-Armour),* and 10 rats received daily injections of 2.5 mg. of Compound E (Cortone).* An additional 15 rats were used as control animals.

At the end of the three week period of hormone administration, all animals were subjected to bile duct cannulation and bile was collected over the ensuing 24 hour interval, during which food was withheld. Samples of plasma were obtained preoperatively and analyzed for concentration of cholesterol; bile samples were analyzed for concentration of cholesterol and bile acids (cholate). All determinations were made according to previously described technics (Byers, Friedman and Michaelis, 1950; Friedman, Byers and Michaelis, 1951).

RESULTS

The results of these studies are presented in Tables 1 and 2. Under the conditions of this experiment, none of the hormonal preparations which

TABLE 1. THE EFFECT OF SEX HORMONES ON 1) PLASMA CHOLESTEROL CONCENTRATION AND 2) BILE CHOLESTEROL AND BILE ACID CONCENTRATION

Hormone administered	No. of rats	Weight (gm.)	Plasma cholesterol concentration (mg./100 ml.)	Bile				
				Daily volume (ml.)	Cholesterol		Cholate	
					Concentration (mg./100 ml.)	Daily output (mg.)	Concentration (mg./100 ml.)	Daily output (mg.)
Extrogen	10	243	54.4	13.3	10.4	1.4	240	30.3
Range:		(203–277)	(36–67)	(11.5–17.0)	(8.7–12.7)	(1.0–1.7)	(194–300)	(22–40)
S.E. Mean:					± 0.4	± .08		
Progesterone	10	308	53.7	14.7	14.7	2.3	235.5	33.5
Range:		(254–337)	(36–68)	(8–19)	(8.4–23.0)	(0.9–3.5)	(174–450)	(25–61)
S.E. Mean:					± 1.5	± .24		
Testosterone	10	300	48.3	14.0	14.3	2.1	219.7	30.3
Range:		(270–320)	(42–62)	(10.5–21.0)	(5.9–27.0)	(1.2–3.8)	(190–253)	(22–40)
S.E. Mean:					± 1.8	± .22		
Control	5	271	44.4	13.0	15.9	2.0	254	33.0
Range:		(248–306)	(32–60)	(11–15)	(14.0–20.0)	(1.5–3.0)	(170–320)	(20–42)
S.E. Mean:					± 1.4	± .25		

were used appeared to have any significant effect upon the plasma cholesterol concentration of the rat. The latter all were in the range of values previously obtained in this laboratory in rats of this strain. Moreover, no significant change in the bile volume or in the daily biliary output of cholic acid was observed in any of the different groups of rats.

* We wish to express our appreciation to the Schering Corporation for supplies of Progynon-B, Proluton, and Oreton; to Merck and Co. for supplies of Cortone Acetate; to Armour Laboratories for supplies of ACTH; and to the Upjohn Co. for supplies of Lipoadrenal Extract.

The biliary concentration and daily output of cholesterol apparently were not affected by the administration of progesterone, testosterone, adrenocortical extract, or compound E (Cortone). On the other hand, significant changes appeared to occur in the rats which received estrogenic and adrenocorticotrophic hormones. Thus, the average biliary cholesterol concentration was 10.4 mg. per 100 cc. in the rats receiving estrogenic hormone, compared to 15.9 mg. per 100 cc. in the control group (Table 1). The average concentration of biliary cholesterol was 19.6 mg. per 100 cc. in the rats given adrenocorticotrophic hormone, compared to an average

TABLE 2. EFFECT OF ACTH AND ADRENOCORTICAL STEROIDS ON 1) BLOOD CHOLESTEROL CONCENTRATION AND 2) BILE CHOLESTEROL AND BILE ACID CONTENT

Hormone administered	No. of rats	Weight (gm.)	Plasma cholesterol concentration (mg./100 ml.)	Bile				
				Daily volume (ml.)	Cholesterol		Bile acid (Cholate)	
					Concentration (mg./100 ml.)	Daily output (mg.)	Concentration (mg./100 ml.)	Daily output (mg.)
ACTH	10	293	51.2	16.4	19.6	3.2	202	32.7
Range:		(259–327)	(44–66)	(11.5–20.0)	(12.6–29.0)	(2.0–4.2)	(160–250)	(29–38)
S.E. Mean:					± 1.7	± .2		
Cortisone	9	265	61.7	16.2	14.8	2.4	186	33.0
Range:		(200–329)	(53–68)	(11.5–20.5)	(9.5–21.0)	(1.7–3.7)	(132–240)	(27–35)
S.E. Mean:					± 1.3	± .22		
Lipoadrenal Extract	10	283	57.4	16.8	16.8	2.8	176.6	28.5
Range:		(246–309)	(50–67)	(10.0–21.5)	(11.3–21.5)	(1.1–6.1)	(110–280)	(22–37)
S.E. Mean:					± 2.4	± .43		
Control	10	265	52.4	13.3	14.4	2.1	254.5	31.2
Range:		(230–298)	(42–70)	(9–18)	(10.0–21.0)	(1.0–3.8)	(210–320)	(28–38)
S.E. Mean:					± 1.1	± .28		

of 14.4 mg. per 100 cc. in their respective control group (Table 2). These differences appeared to be statistically significant.

DISCUSSION

Previous studies from this laboratory clearly have shown that the biliary concentration of cholesterol may be regarded as an index of the rate of hepatic cholesterol synthesis in the rat (Byers and Friedman, 1952). Moreover, the marked increase and decrease in biliary cholesterol content which were found in hyper- and hypothyroid rats, respectively (Rosenman *et al.*, 1951, 1952) have been shown to parallel closely the demonstrated alteration in their respective rates of synthesis of cholesterol, as shown by physiological and by tracer studies (Rosenman *et al.*, 1952; Byers *et al.*, in press). In the present investigation, similar changes in biliary cholesterol concentration were sought as evidence of any significant effect of the various hormonal substances upon the rate of hepatic synthesis of cholesterol in the rat.

It might have been anticipated that certain of the preparations which

were used would have induced changes in biliary cholesterol concentration. Cholesterol has been shown to be a precursor of various steroid hormones (Zaffaroni, Hechter and Pincus, 1951; Jailer, 1949; Bloch, 1945). Moreover, a number of studies might be cited which purport to show a controlling influence of adrenal steroids upon fat metabolism (Zilversmit, Stern and Overman, 1951; Li, Simpson and Evans, 1949; Ingle, 1943; Adlersberg *et al.*, 1950, 1951). This subject was extensively reviewed by Ingle (1943) whose considered opinion was that a primary effect of the adrenals upon fat metabolism has never clearly been demonstrated. On the other hand, administration of ACTH or Compound E in humans is claimed early to reduce the serum cholesterol level (Conn *et al.*, 1950; Dustan, Corcoran, Taylor and Page, 1951), with an increase after prolonged therapy (Adlersberg, Schaeffer and Dritch, 1950, 1951). Similar effects are held for Compound E in the rabbit (Kobernick and More, 1950; Adlersberg, Drachman and Schaeffer, 1951), although no change was observed in the dog. The changes described in humans may be related to the utilization of cholesterol as a precursor of adrenal steroids (Conn *et al.*, 1950; Zaffaroni *et al.*, 1951), or possibly to the altered thyroid status which may follow prolonged administration of these substances (Wolfson *et al.*, 1950).

No definite effect upon serum lipids has been noted with administered androgenic substances (Enterman, Lorenz and Chaikoff, 1940). Estrogens, however, induce a definite hyperlipemia in fowl (Horlick and Katz, 1948; Riddle and Sensum, 1939), an effect not observed in the rat (Horlick and Katz, 1948). In human subjects estrogens were found to depress serum cholesterol levels (Eilert, 1949). Cyclic variations of serum cholesterol also have been described in women (Okey and Baden, 1927), with significant changes occuring during the menses and pregnancy (Slemons and Stander, 1923; Boyd, 1934), periods during which estrogen and progesterone levels are changed. Furthermore, the fall in the blood level of these hormones, which occurs at the end of pregnancy (Smith and Smith, 1947), is associated with a decrease in the serum cholesterol concentration (Granirer, 1950). On the other hand, estrogens failed to affect cholesterol levels in hyperthyroid states (Farbman, 1944).

In reviewing previous studies of the influence of various hormones upon cholesterol metabolism, there is no unequivocal evidence demonstrating a primary effect of androgens, progesterone, or adrenocortical steroids. The values for plasma cholesterol which were obtained following hormonal therapy in our experiements in rats indicate that, under the condition of this study, none of the preparations used had any significant effect. Moreover, in using the biliary cholesterol concentration as an index of the rate of hepatic cholesterol synthesis (Byers and Friedman, 1952), only the

estrogenic and adrenocorticotrophic hormones appeared to have any significant effect. In this regard, the results suggest that hepatic synthesis of cholesterol is decreased in rats given estrogenic hormone and somewhat increased in rats given adrenocorticotrophic hormone.

SUMMARY

The biliary concentration of cholesterol previously was shown to be an indicator of hepatic synthesis of cholesterol in rats. This method was used to study the influence of adrenocorticotrophic hormone, adrenocortical steroids, and the sex hormones. The results suggest that hepatic synthesis of cholesterol in rats is depressed by estrogenic hormone and augmented by administration of ACTH. None of the preparations used appeared to have any effect upon bile volume output, biliary excretion of cholate, or upon plasma cholesterol levels.

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SOME DIFFERENCES BETWEEN TWO STRAINS OF RATS DEVELOPED BY SELECTION TO DIFFER IN THEIR RESPONSE TO EQUINE GONADOTROPHIN[1]

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SOME of the causes of variation in the response of the ovaries of 21-day-old rats to a standard dose of equine gonadotrophin have been reported by Chapman (1946). Heredity was estimated on his random-bred stock as responsible for 40% of the variance in the weight of the stimulated ovaries after the variability due to body weight and to temporal factors was eliminated. Selection and inbreeding experiments have since been carried out on the descendents of this stock to develop lines that differ markedly in their quantitative response to the gonadotrophic hormone (Kyle and Chapman, 1948).

The ability of a rat ovary to respond to gonadotrophic stimulation is associated with the presence of vesicular follicles (Corey, 1930 and Hargitt, 1930). It may be suspected that the larger the number of vesicular follicles present, within limits, the greater will be the response to a given amount of hormone and that differences in number of vesicular follicles might account for differences between lines in the degree of this response.

It was thought that a rapid method of surveying the relative follicular development in a number of animals might be through the determination of the percentage of water or inversely the percentage of solids in the ovaries. A negative association had been shown between the weight of stimulated ovary and percentage of ovarian solids (Casida, Meyer and McShan, 1943). Further, the percentage dry weight determined on follicular fluid and on ovarian stroma from the ovaries of cows, in which mechanical separation of these components can be made readily, had been found to be 10% or less for the former and 20% or more for the latter (unpublished data, University of Wisconsin).

Differences in numbers of vesicular follicles per ovary might exist either

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as differences per unit mass of the ovary or as differences in quantity of ovarian tissue with equal concentrations of vesicular follicles per unit of mass. If the differences between two lines were traceable to differences in mass of ovary of equal quality, so far as follicular development is concerned, then no difference should be found in the percentage of solids in the ovaries of rats from the two lines. If, on the other hand, there was a different number of follicles of equal size per unit of ovary in the two strains or a difference in size of follicle not compensated for by number of follicles then one might expect a difference in percentage of solids.

The hypothesis that differences between lines in their response to a standard dose of gonadotrophic hormone are dependent upon the initial degree of follicular development in the ovaries at the time of treatment was tested by determining the percentage of ovarian solids in two such lines inbred 70–90%. In addition, the characters, body weight, age at vaginal opening and litter size were examined to learn if they had been affected by the selection exercised in bringing about the difference in ovarian response.

MATERIALS AND METHODS

Studies were made on 20 pairs of full sisters and their dams from the L4, low-response, line and of 19 pairs of full sisters and their dams from the H7, high-response, line. One member of each pair of sisters was injected at 21 days of age with a standard dose of equine-gonadotrophin (1 Cartland-Nelson unit) and autopsied at 25 days. (Selection of breeding animals in developing these lines was on the basis of ovarian response to such treatment in injected sisters and progeny.) The other member of each pair was autopsied at 21 days. The fresh ovarian weight and the percentage of dry ovarian solids were determined on each pair of ovaries, (from both injected and uninjected rats) as was also the body weight at 21 days of age. Data on the dams included age at vaginal opening, body weight at 13 weeks and size of first litter. Matings were begun regularly at 13 weeks of age.

RESULTS AND DISCUSSION

The results of the selection program that preceded this study are indicated by the difference in the ovary weights of the treated rats from the H7 and the L4 strains (table 1). The difference of 42.8 mg. is highly significant statistically and is equal to approximately one-half the weight of the ovaries of the less responsive strain.

The selection which had been practiced in developing these two strains had been done without reference to any ovarian characteristics other than weight of ovaries after hormonal stimulation. The difference between strains in the weights of the ovaries from the untreated rats offers an explanation for much of the previously observed difference in the treated animals. The difference of 3.35 mg. between the weights of the ovaries of the two strains at 21 days is approximately 40% of the weight of the untreated ovaries from the lower-response strain. This is approximately equivalent

to the proportional difference after treatment. In other words, one milligram of untreated ovary in both the L4 and H7 rats produces approximately the same amount of treated ovary, viz., 9.5–10.5 mg.

It was assumed for purposes of comparing the strains that a lower percentage of solids in untreated rats indicates a higher concentration of vesicular follicles and a greater potentiality of the ovaries to respond to injected hormone; no evidence for explaining the difference in response on this basis, however, was found between the two lines (H7 − L4 = 0.72%; P≅0.30). Further, a difference in percentage of solids might indicate a difference in the amount of gonadotrophic hormone acting in the two lines at 21 days. This is assuming that endogenous hormone lowers the solids percentage as does exogenous hormone. The injected hormone, as antici-

TABLE 1. COMPARISON OF H7 AND L4 INBRED LINES OF RATS

Character	Animal	H7 "High	L4 "Low	Mean difference
Ovary wt.—mg.	Treated	122.2	79.4	42.8*
Ovary wt.—mg.	Untreated	11.64	8.29	3.35*
Ovarian solids—%	Untreated	16.90	16.18	0.72
Ovarian solids—%	Treated	14.06	14.03	0.03
Age at vaginal opening—days	Dams	48.16	42.15	6.01*
Body wt., 21 days—gms.	Treated and Untreated	42.11	40.00	2.11
Body wt., 13 weeks—gms.	Dams	206.4	158.0	48.4*
1st litter size—no.	Dams	6.68	6.20	0.48

* $P < 0.01$.

pated, lowered the solids content in the H7 and L4 lines by $2.84 \pm 0.62\%$ and $2.15 \pm 0.68\%$, respectively. The insignificant difference in percentage of solids between the two lines without treatment, does not give support to the hypothesis of an initial difference in their own gonadotrophic activity.

If the greater ovarian development in the untreated H7 were associated with some aspect of physiological maturity of the follicles not reflected in proportion of follicular fluid in the ovary one might expect it to be shown in the earliness of sexual maturity of females of this strain. The dams of the rats just described were examined for the age at first vaginal opening. A significant difference of 6.01 days existed between the means of the two strains, but the H7 strain matured at the greater age. Cole and Casady (1947) also studied two inbred lines of rats that differed in the weights of the immature ovaries. They, however, found that the strain with the larger ovaries matured at the earlier age. The results of the present study indicate that this is not a necessary physiological association.

Other findings of Cole and Casady were that the rats with the heavier

ovaries also were heavier in body weight and produced larger litters. The body weights at 21 days of age in the present study differed by 2.11 gms. in favor of the H7 ($P \cong 0.15$). The dams at 13 weeks of age gave definite evidence of a strain difference amounting to 48.4 gms., or approximately 30% of the L4, the lighter weight strain. The average litter size of the H7 strain was insignificantly larger (0.48 of a pup) than the L4. Thus, there was confirmation of the above results so far as body weight was concerned, but little or no evidence of agreement on the basis of size of first litter.

The difference in the two strains of rats in the response of their ovaries to gonadotrophic hormone may, for the purposes of further study, be considered traceable to the presence of different amounts of ovarian tissue upon which the hormone can act. The character of the tissue present (concentration of vesicular follicles) however, appears to be essentially the same. The difference in the ovaries of the two lines may be due more to general somatic growth than to differences in endogenous gonadotrophic hormone that may have acted upon the ovaries.

SUMMARY

A comparison has been made of two inbred lines of rats developed by generations of selection to differ in the response of their ovaries to a standard dose of equine gonadotrophin. Statistically significant differences between the respective mean values for the high and low-response lines were found in the following characteristics: ovary weight—25-day-old treated rats, 122.2 and 79.4 mg., 21-day-old untreated rats, 11.64 and 8.29 mg.; age at vaginal opening, 48.16 and 42.15 days; body weight at 13 weeks, 206.4 and 158.0 gms. Insignificant differences were found between the respective means of high and low lines for the following characteristics: ovarian solids—25-day-old treated rats, 14.06 and 14.03%, 21-day-old untreated rats, 16.90 and 16.18%; body weight at 21 days, 42.11 and 40.00 gm.; number of young in first litter, 6.68 and 6.20.

These findings suggest that the differences in response of the two lines is due largely to a difference in the amount of ovarian tissue at the time of treatment.

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NOTES AND COMMENTS

PINEALECTOMY VS. PINEAL INJECTION IN THE YOUNG COCKEREL

The pineal body remains an organ of doubtful endocrine function. Examination of review articles (Anderson and Wolf, 1934, Krabbe, 1923, Simonnet and Thieblot, 1951, Simonnet and Sternberg, 1951, Sternberg, 1951) discloses that the following conflicting claims are found in the literature.

1. Pinealectomy has been claimed to cause any one of 3 effects on the gonads: Hypertrophy, or atrophy, or no effect.
2. The same claims have been made following addition of pineal material by implantation or injection, hypertrophy, or atrophy, or no effect on gonad weight.

One of these claims (Shellabarger and Breneman, 1950) reports that pinealectomy produces a decreased testis weight in 20-day-old cockerels. In order to extend this observation, the following experiment was designed to make use of comparisons between pinealectomized animals, pineal injected animals, and pinealectomized animals receiving pineal injections.

MATERIALS AND METHODS

White Leghorn males were pinealectomized and sham operations were performed 2 days post-hatching. Only data obtained from chicks in which macroscopical or histological examination of the site of operation revealed no trace of the pineal body were considered here. One gram of acetone dried lyophilized beef pineal[1] was mixed with 40 cc of distilled water and stored in the refrigerator at 8° C. Two tenths cc of the solution was injected daily and when the mixture was exhausted a new supply was made up. Thus each chick received approximately 5 mg. of pineal material per day from the 5th to 19th day post-hatching. A similar amount of acetone dried, beef brain matter was used as a control injection. The birds were autopsied on the 20th day post-hatching.

RESULTS AND DISCUSSION

The testis weights are given in table 1 and an analysis of variance is presented in

TABLE 1. MEAN TESTIS WEIGHTS, NUMBER OF ANIMALS IN THE EXPERIMENTAL GROUPS, AND SUM OF X'S. 20-DAY MALE WHITE LEGHORNS

	Sham operated	Pinealectomized	
No injection	36	26	No.
	1837	1151	Sum of X's
	51 mg.	44 mg.	Mean
Brain injection	26	24	No.
	1169	1049	Sum of X's
	48	44	Mean
Pineal injection	32	26	No.
	1771	1299	Sum of X's
	55	50	Mean

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[1] The author wishes to thank J. H. Strache, Armour and Company for providing the lyophilized beef pineal material.

table 2. All statistical methods are those routinely used (Snedecor, 1948). The results of this analysis of variance are interpreted as follows: Both operation and injection had a significant effect, but in opposite directions; there was a significant difference between pineal injection and the combined treatment of brain injection and no injection; no difference could be demonstrated between brain injection and no injection; and no significance could be demonstrated in regard to interaction. Thus it is felt that the data support the suggestion that during the first 20 days the absence of the pineal body has an inhibitory effect on testis growth. Although the differences between the testis weights are quite small, the use of sufficient numbers of experimental animals and an

TABLE 2. ANALYSIS OF VARIANCE OF TESTIS WEIGHTS

Source of variation	Degrees of freedom	Sum of squares	Mean square
Operation	1	960	960*
Injection	2	1995	998*
brain injection vs. no injection	1	406	406
pineal injection vs. brain+no injection	1	1589	795*
Interaction	2	166	83
Individuals	164	17579	107

* P beyond the 1% level.

TABLE 3. RESULTS OF ASSAY OF PITUITARIES. FIGURES GIVEN REPRESENT AVERAGE VALUES OF TESTIS WEIGHTS OF THE ASSAY BIRDS, EACH FIGURE REPRESENTS AT LEAST 12 ASSAY ANIMALS

	Sham	Pinealectomized	Water injected assay animals
No injection	9.2 mg.	6.4 mg.	5.6 mg.
Brain injection	7.8	7.2	
Pineal injection	7.1	7.4	

adequate number of control animals suggests that a true endocrine response must be considered. The possibility that the difference in testis weights is due to a modification of gonadotropic potency of the pituitary was tested by the method regularly used in our laboratory (Breneman and Mason, 1951). The results of the pituitary assay are presented in table 3. It is of interest to note that the gonadotropic potency of the pituitary was significantly changed in only one instance (sham vs. pinealectomized, t-value 3.696, P value beyond the 1 per cent level).

SUMMARY

Pinealectomy resulted in inhibition of testis growth, the injection of pineal material into non-operated chicks resulted in an increased testis growth, and the injection of pineal material into pinealectomized chicks restored the testis weights to normal.

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THYROID FUNCTION AS RELATED TO THE GROWTH OF TADPOLES BEFORE METAMORPHOSIS

Evidence for the function of the anuran thyroid gland before metamorphosis has been presented by Hoskins and Hoskins (1919), Etkin (1935), Gorbmann and Evans (1941) and Steinmetz (1950, 1951). It has been claimed, however, that the definite onset of thyroid function does not occur until the beginning of metamorphosis (Moore, 1950). It is the purpose of this note to present some observations on the growth and respiration of premetamorphic *Rana clamitans* larvae treated with 2-thiouracil, thiourea, and thyroxine which indicate that thyroid secretion plays a definite role in anuran physiology before metamorphosis.

METHODS

Eggs obtained from freshly captured frogs were fertilized by the method of Rugh (1934) and divided into 4 groups. All animals were reared in aerated tap water. Group 1 was reared in finger bowls on a diet of Pablum (Steinmetz, 1950) in the following concentrations of 2-thiouracil: 0.015%, 0.030% and 0.045%. Group 2, reared in finger bowls on a diet of Pablum and commercial dog food, was treated with 0.045% 2-thiouracil. Group 3 was grown in ten liter aquaria on the same diet as group 2 and was treated with 0.03% thiourea. Group 4 was reared as group 1 with the following treatments: 0.03% 2-thiouracil, 1 μg./liter thyroxine and 5 μg./liter thyroxine. Untreated control series were reared with all groups. All antithyroid treatments were begun on the 11th day after fertilization and thyroxine treatments were begun on the 29th day after fertilization. Micro-Kjeldahl determinations of total nitrogen were made by the method of Niederl and Niederl (1942) utilizing the digestion mixture of Brüel, Holter, Linderstrøm-Lang and Rozits (1947). Determinations of fat content were made on samples of ten larvae in a standard Soxhlet apparatus. Oxygen consumption of individual larvae was determined utilizing the Barcroft-Warburg apparatus. The reaction vessels were shaken at 120 oscillations/minute at 28.35°C. Statistical significance of all differences between the means of control and experimental series was evaluated by the "t" test (Snedecor, 1945).

RESULTS AND DISCUSSION

The results of the various determinations are summarized in tables 1 and 2. The measurements of wet weight, dry weight, total nitrogen and total fat show that treatment with antithyroid compounds results in a more rapid growth of the treated larvae. These results are the reverse of those obtained by thyroxine treatment. The differences in growth are also reflected in the oxygen consumption of the treated larvae; the larger, antithyroid treated larvae consume more oxygen per unit time than the controls, while the smaller, thyroxine treated larvae consume less. These may be misleading results

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TABLE 1. A SUMMARY OF THE DATA (MEANS) SHOWING THE EFFECTS OF THIOURACIL, THIOUREA AND THYROXINE ON THE GROWTH AND RESPIRATION OF PREMETAMORPHIC *Rana Clamitans* LARVAE

Group	Age	Treatment	n	Wet wt. mg.	Dry wt. mg.	Total N mg.	QO_2[1]	% N Wet wt.	% N Dry wt.	QO_2[1] per mg. N	QO_2[1] per mg. dry wt.
4	16	Control	5	16.8	1.4	.1304	—	.796	9.88	—	—
4	16	30 mg. % Thiouracil	5	16.3	1.3	.1347	—	.826	10.85	—	—
4	24	Control	10	23.3	2.1	.1874	—	.804	8.91	—	—
4	24	30 mg. % Thiouracil	10	24.4	1.8	.2089	—	.820	12.12	—	—
4	32	Control	10	27.3	2.6	.2365	—	.862	9.46	—	—
4	32	30 mg. % Thiouracil	10	29.9	2.4	.2550	—	.854	10.54	—	—
4	40	Control	10	31.6	3.0	.2704	23.28	.857	9.16	85.19	7.98
4	40	30 mg. % Thiouracil	10	35.0	3.3	.3246*	29.77	.934	10.14	100.24	9.18
4	40	5 μg./l Thyroxine	10	25.0*	2.7	.2113†	22.84	.843	7.79†	108.66	8.05
1	49	Control	10	30.6	1.9	.2164	18.36	.705	11.86	83.30	9.84
1	49	30 mg. % Thiouracil	10	42.7†	3.0†	.3307†	24.87*	.778	11.15	84.82	8.24
3	53	Control	15	27.3	2.1	.1998	11.44	.733	9.90	56.06	5.52
3	53	30 mg. % Thiourea	15	46.2†	4.0†	.4242†	26.43†	.912†	10.69	61.44	6.59†
4	53	Control	10	33.0	2.9	.3046	22.09	.920	10.55	76.77	7.88
4	53	30 mg. % Thiouracil	10	49.2†	4.2†	.4408	31.52	.900	10.53	72.39	7.37
4	53	5 μg./l Thyroxine	10	23.8†	2.0†	.1943†	15.82†	.814	9.92	82.74	8.10
1	67	Control	9	35.2	2.9	.2721	20.15	.79	9.52	73.73	7.12
1	67	15 mg. % Thiouracil	8	50.4*	3.8†	.4143†	27.02	.791	10.77	69.00	7.15
1	67	30 mg. % Thiouracil	9	64.9†	4.6†	.5130†	31.05†	.792	11.21*	60.58	6.75
1	67	45 mg. % Thiouracil	8	76.5†	6.6†	.5821†	38.58†	.758	8.75	66.25	5.86
2	81	Control	9	67.3	5.5	.6060	39.64	.892	11.14	64.52	7.12
2	81	45 mg. % Thiouracil	9	110.0†	9.3†	.9436†	62.90†	.857	10.07	67.29	6.75

[1] QO_2 values are calculated as μl. O_2 consumed at STP in a two hour period.
* Indicates 5% and † indicates 1% level of significance.

TABLE 2. THE EFFECTS OF THIOURACIL, THIOUREA AND THYROXINE ON THE TOTAL AND RELATIVE AMOUNT OF FAT IN PREMETAMORPHIC *R. clamitans* LARVAE

Group	Treatment	Age	n[1]	Wet wt.[2] mg.	Dry wt.[2] mg.	Total[2] Fat mg.	% Dry wt. / Wet wt.	% Fat / Wet wt.	% Fat / Dry wt.
4	Control	67	6	452	33.0	1.82	7.48	.403	5.4
4	30 mg. % Thiouracil	67	6	609†	45.8*	2.48†	7.51	.408	5.4
4	5 μg./l. Thyroxine	67	7	290†	21.0*	1.23†	7.25	.419	5.8
3	Control	53	5	319	20.4	1.36	6.36	.418	6.6
3	30 mg. % Thiourea	53	5	430†	32.1*	2.02	7.46	.461	6.5
1	Control	79	2	458	32.8	2.35	7.16	.449	7.0
1	30 mg. % Thiouracil	79	2	643	46.2	3.40	7.18	.537	7.5

[1] n = the number of groups of ten larvae used.
[2] The figures given represent the group means rather than the individual means.
* Indicates 5% and † indicates 1% level of significance.

inasmuch as the oxygen consumption of a larva in an oscillating reaction vessel may not be representative of the oxygen consumption of the larva in a normal environment. The present data agree with previous experiments in that significant differences were not observed in the antithyroid treated larvae until after 28 days of age. This is interpreted as indicating that the thyroid hormone does not begin to function effectively until 3 to 4 weeks after hatching. The results of thyroxine treatment support the view that a low level of thyroid secretion can be maintained during the premetamorphic period inasmuch as the 1 μg./liter dosage had a marked effect on growth (mean body weights at 67 days of age: 60 controls = 45.2 mg., 40 experimental = 29.8 mg.†), but no detected metamorphosis-inducing capacity. The 5 μg./liter dosage, however, resulted in some hindlimb bud development in addition to the effects on growth.

When the experimental differences are compared on a relative basis, *i.e.* %N/wet wt.,

%N/dry wt., QO_2/mg.N, QO_2/mg. dry wt., %dry wt./wet wt., %fat/wet wt. and %fat/dry wt., no consistent differences which might be attributed to the treatments are observed. It also may be noted that variations in diet, environment and chemical composition of the antithyroid agent tested did not significantly alter the character of any of the responses measured. All the data presented are interpreted as supporting the general hypothesis that the anuran thyroid gland begins active function by the 3rd or 4th week after hatching and that its secretion acts to control the rate of the normal growth process.

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